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METHODS FOR TREATING DRUG ADDICTION

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METHODS FOR TREATING DRUG ADDICTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application is a National Stage of US application number PCT/US02/11094, filed April 4, 2002; said application claims the benefit under 35 USC § 119(e) of U.S. provisional application No. 60/281,440 filed April 4, 2001. The aforementioned applications are incorporated herein by reference for all purposes.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[2] The present invention relates to the identification of differentially expressed genes in the brain that are involved in behavior associated with cocaine addiction. More particularly, the present invention relates to methods of identifying and using candidate agents to treat cocaine addiction based upon these genes.

2. Description of the Related Art

[3] Drug and alcohol addictions are mental illnesses that exact an enormous social and economic cost from society. Although biomedical research has made tremendous advances in our understanding of how drugs affect the brain, very little of this information has translated into effective treatment strategies. This problem is particularly troublesome for cocaine addiction, where no effective treatments currently exist. Although many cocaine addicts can abstain from drug use for short periods of time, relapse rates at longer periods of abstinence are remarkably high, sometimes exceeding 90% (see Leshner, "Addiction Is a Brain Disease, and It Matters", Science, Vol. 278, pp. 45-47 (1997)).

[4] Progress in treating cocaine addiction has been hampered by the failure of animal models to target the primary behavioral disturbance, i.e., the increased propensity for relapse following prolonged periods of abstinence. Recently, the realization of this problem has led investigators to develop new animal models of drug craving in attempts to understand the underlying neurobiological mechanisms that trigger relapse to drug-seeking behavior, and to develop more effective treatment. In these studies, laboratory measures of

"cocaine-seeking behavior" provide an objective measure of operant events such as lever-press responses that represent approach behavior analogous to relapse. In these studies, the level of drug-seeking behavior is indicated by the amount of effort (lever-pressing) exerted by animals to self-administer the drug. Importantly, this cocaine-seeking behavior is tested in the absence of drug reinforcement, because the reinforcing and rate-limiting effects of drugs can obscure the true incentive motivational state of the animals. Cocaine-seeking behavior can be measured by the magnitude and persistence of drug-paired lever responding during extinction testing, and by "reinstatement" of this responding following extinction. Either of these measures are thought to reflect the propensity for relapse in humans. Another advantage of these paradigms is that they can be tested during prolonged periods of forced abstinence. In contrast, subjective measures of drug craving in humans can be confounded by the subjective nature of self reports, and contextual differences between laboratory settings and the environment where humans routinely take drugs (see Tiffany et al., "The Development of a Cocaine Craving Questionnaire", Drug Alcohol Depend., Vol. 34, pp. 19-28 (1993)).

[5] Generally, there are only three stimuli known to reinstate drug-seeking behavior in animals following extinction of drug-self-administration. These stimuli consist of drug-associated (conditioned) cues, stress and low "priming" doses of the self-administered drug itself (for review, see Self et al., "Relapse to Drug Seeking: Neural and Molecular Mechanisms", Drug Alcohol Depend., Vol. 51, pp. 49-60 (1998)). Since all three of these stimuli also trigger drug craving in human drug abusers (see Jaffe et al., "Cocaine-Induced Cocaine Craving", Psychopharmacology, Vol. 97, pp. 59-64 (1989); Robbins et al., "Relationships Among Physiological and Self-Report Responses Produced by Cocaine-Related Cues", Addictive, Vol. 22, pp. 157-167 (1997); and Sinha et al., "Stress Induced Craving and Stress Response in Cocaine Dependent Individuals", Pschyopharmacology, Vol. 142, pp. 343-351 (1999) reinstatement of drug-seeking behavior in animals may represent a valid model of human drug craving. One caveat is that human drug addicts rarely, if ever, experience extinction conditions prior to relapse, but the striking concordance of reinstating stimuli in animals, and triggers of craving in humans, suggests that similar neurobiological processes are involved in both reinstatement and craving.

[6] Figure 1 depicts some of the primary pathways whereby stress, priming injections of drugs, and drug-associated cues are thought to induce relapse to drug-seeking

behavior based on an evolving literature. There is a growing evidence that these stimuli all induce relapse, at least in part, by their ability to elevate dopamine levels in the nucleus accumbens (NAc). Thus, the NAc may be a critical neural substrate for relapse to drug seeking, in addition to its well-characterized role in drug reward. For example, abused drugs which elevate NAc dopamine levels also reinstate cocaine- and heroin-seeking behavior, while abused drugs like barbiturates that do not elevate NAc dopamine levels also fail to reinstate this behavior (reviewed by Self et al., *supra*). Similarly, infusion of drugs into brain regions where they activate NAc dopamine release reinstates cocaine- and heroin-seeking behavior, where infusion into regions where they do not is without effect.

[7] Although it has not been clearly resolved, cue- and stress-induced reinstatement of drug-seeking behavior may involve both dopamine-dependent and dopamine-independent neural substrates (reviewed by Self et al., *supra*). An area of excitatory convergence is the NAc, where excitatory inputs from the prefrontal cortex (PfC), basolateral amygdala (BLA), and subiculum innervate medium spiny neurons receiving dopamine inputs from the ventral tegmental area (VTA). Excitatory neurotransmission in the NAc also has been implicated in reinstatement of cocaine-seeking behavior (see Cornish, et al., "A Role for Nucleus Accumbens Glutamate Transmission in the Relapse to Cocaine-Seeking Behavior", Neuroscience, Vol. 93, pp. 1359-1367 (1999)). Together, these brain regions all form a complex circuit with primary sites of convergence in both the VTA and NAc of the mesolimbic dopamine system, as depicted in Figure 1.

[8] These studies highlight new and important information on the neural mechanisms of drug craving and relapse to drug seeking. Given that drug-seeking and drug craving can persist (or increase) despite long periods of abstinence, many current theories suggest that relatively long-term neuroadaptations in limbic brain regions associated with drug-seeking behavior underlie the propensity for relapse in addicted individuals. Most of these theories focus on pharmacological neuroadaptations directly produced by repeated drug exposure, leading to the phenomena of tolerance and sensitization (see Koob et al., "Drug Abuse: Hedonic Homeostatic Dysregulation", Science, Vol. 278, pp. 52-58 (1997) and Nestler et al., "Molecular and Cellular Basis of Addiction", Science, Vol. 278, pp. 58-63 (1997)). However, there is little evidence that most neuroadaptations persist during prolonged periods of abstinence (see White et al., "Neuroadaptions Involved in Amphetamine and Cocaine Addiction", Drug Alc. Dep., Vol. 51, pp. 141-153 (1998)), and,

thus, they cannot fully account for the propensity for relapse at these later time points. A major gap in our current knowledge is identifying stable neuroadaptations that underlie persistent drug craving in prolonged abstinence.

[9] Recently, a behavioral paradigm in rats has been developed that models persistent craving for cocaine during prolonged abstinence. In fact, rats actually show increased levels of cocaine-seeking behavior as abstinence proceeds, a phenomenon also recently reported by Neisewander and colleagues (see Tran-Nguyen et al., "Time-Dependent Changes in Cocaine-Seeking Behavior and Extracellular Dopamine Levels in The Amygdala During Cocaine Withdrawal", Neuropsychopharmacology, Vol. 19, pp. 48-59 (1998)). In this model, the level of cocaine-seeking behavior progressively increases from 1-6 weeks of forced abstinence from cocaine self-administration. The model is referred to as the "Cocaine Abstinence Effect", and is thought to reflect time-dependent increases in cocaine craving that lead to increased relapse rates during prolonged abstinence. The model also represents the phenomenon of incentive sensitization, whereby drug-associated stimuli (environmental context, conditioned cues) show enhanced ability to stimulate craving as abstinence proceeds (see Robinson et al., "The Neural Basis of Drug Craving: An Incentive-Sensitization Theory of Addiction", Brain Res. Rev., Vol. 18, pp. 247-291 (1993)).

[10] In this model, rats are allowed to acquire intravenous cocaine self-administration on a fixed ratio 1:time-out 15-second schedule of reinforcement for 4 hours/day. Following 12 days of cocaine self-administration, different periods of forced abstinence are imposed whereby animals remain in their home cages, and are not allowed access to the self-administration test chambers. After a given period of abstinence, the rats are returned to the self-administration chambers, and the degree of drug-seeking behavior is measured by the number of non-reinforced responses at the drug-paired lever during extinction testing. Figure 2 shows that cocaine-seeking behavior is approximately tripled when rats are returned to the test chambers during the third and sixth week of abstinence, relative to rats returned during their first week of abstinence. Six weeks of abstinence also produces more persistent cocaine-seeking behavior over the first few days of testing. By the sixth day of extinction testing, all three groups have extinguished to similar levels.

[11] Figure 3A shows time-dependent changes in the initial level of cocaine-seeking behavior when rats are first returned to the self-administration test chambers following forced abstinence. Rats tested after 2 and 5 weeks of forced abstinence exhibit 5-

to 6-fold greater levels of drug-seeking behavior than at 1 day of abstinence. At the end of extinction testing, rats were tested for cue-induced reinstatement of cocaine-seeking behavior. In this test, cues specifically associated with the 10-second cocaine infusions during self-administration (house light off; lever cue light on; pump noise, vehicle infusion) were presented every 2 minutes for the final hour of the extinction/reinstatement test session. Figure 3B shows that the cocaine abstinence effect is still evident following extinction testing, but only in the group tested during their sixth week of forced abstinence. Thus, cues specifically associated with cocaine infusions during self-administration induced greater reinstatement of responding at 6 weeks of abstinence than at 1 week of abstinence. Moreover, extinction training failed to completely reverse the Cocaine Abstinence Effect in this 6-week group, although cue-induced reinstatement at 3 weeks abstinence failed to differ as in extinction testing.

[12] The Cocaine Abstinence Effect suggests that the incentive motivational effects of the drug-paired environment (extinction), and cocaine-associated cues (reinstatement), gain motivational salience with prolonged abstinence from cocaine. In contrast, pharmacological models of drug addiction and dependence suggest that drug craving would be maximal during early abstinence periods, when withdrawal symptoms also are maximal, and diminish as withdrawal effects wane over time (see Koob et al., *supra*).

[13] The model closely parallels a similar effect of prolonged abstinence from chronic alcohol consumption known as the "alcohol deprivation effect" (see Sinclair, "The Alcohol-Deprivation Effect. Influence of various factors.", Quarterly Journal of Studies on Alcohol, Vol. 33, pp. 769-782 (1972)), although it differs by measuring drug-seeking behavior rather than drug intake. This feature represents an important advantage over models of drug intake, because acute effects of drugs following abstinence could obscure certain biochemical measures that correlate with time-dependent increases in cocaine-seeking, and the response rate-limiting effects of drugs could alter behavioral measures of drug seeking. As cited above, at least one other group has published the phenomenon of time-dependent increases in cocaine seeking using similar (2 and 4 weeks) periods of forced abstinence (see Tran-Nguyen et al., *supra*). This study found that the behavioral effects also were associated with increased basal dopamine levels in the central nucleus (CeA) of the amygdala, and greater increases in dopamine release when animals were first returned to the self-administration chambers during extinction testing.

[14] The "Cocaine Abstinence Effect" animal model is particularly useful in understanding the underlying biochemical neuroadaptations that trigger relapse to drug-seeking behavior. Accordingly, the use of this model to identify changes in gene expression that coincide with time-dependent increases in cocaine-seeking behavior and extinction training in rats, would aid in identifying potential therapeutic targets and therapeutic agents for use in treating cocaine addiction.

SUMMARY OF THE INVENTION

[15] The present invention is based on the identification of genes found in particular brain regions of rats that are modulated by behavior associated with cocaine addiction and extinction training. The genes have been identified by using a behavior animal model of cocaine addiction combined with oligonucleotide array profiling techniques. In particular, the present invention is directed to methods for inhibiting behavior associated with cocaine addiction in a subject such as a mammal suffering from cocaine addiction, and methods for identifying candidate agents useful in inhibiting behavior associated with cocaine addiction, using these genes.

[16] In some embodiments, the invention provides methods for inhibiting addiction-related behavior in a subject suffering from cocaine addiction. These methods involve administering to the subject a therapeutically effective amount of a therapeutic agent which has the ability to modulate the level of activity of a polypeptide encoded by at least one gene identified in one or more of Tables 1-15. The activity of the polypeptide can be modulated by, for example, increasing or decreasing the level of expression of a gene that encodes the polypeptide, the level at which a transcript is translated or maintained in a cell, or by increasing or decreasing the enzymatic activity, binding ability, or other property of the polypeptide itself.

[17] The invention also provides methods of inhibiting addiction-related behavior in a subject suffering from cocaine addiction that involve administering to the subject a therapeutically effective amount of a therapeutic agent which has the ability to decrease transcription/translation of, or decrease the activity of a protein encoded by, at least one gene that encodes a polypeptide selected from the group consisting of hypertension-regulated vascular factor, myelin-associated basic protein, PB cadherin, calcitonin receptor, melanocortin 4 receptor, ALK-7 kinase, and retroposon.

[18] Also provided are methods of inhibiting addiction-related behavior in a subject suffering from cocaine addiction that involve administering to the subject a therapeutically effective amount of an agonist that activates a protein selected from the group consisting of GABA-B receptor subunit gb2, cell adhesion-like molecule, bos taurus-like neuronal axonal protein, a polypeptide similar to mouse chemokine-like factor, FRA-2, a protein similar to human oxygen-regulated protein, a protein similar to mouse mrg1 protein, pentraxin, malic enzyme, olfactomedin-related protein, arc-growth factor, protein tyrosine phosphatase, krox, neuritin, microtubule-associated protein 2d, and CB1 cannabinoid receptor.

[19] Another aspect of the invention provides methods for identifying an agent to be tested for an ability to prevent or inhibit cocaine addiction-related behavior. These methods can involve: a) combining in a reaction mixture a candidate agent with a protein encoded by a gene identified in Tables 1-15; and b) determining whether the candidate agent binds to and/or modulates activity of the protein.

[20] In some embodiments, these methods can further involve adding to the reaction mixture a competitor molecule that competes with binding of the candidate agent to the protein, either prior to or subsequent to combining the protein with the candidate agent.

[21] In other embodiments, the methods further involve: c) administering the candidate agent identified in b) to a cocaine-addicted subject or brain cells of a cocaine-addicted subject, wherein the cocaine-addicted subject is undergoing withdrawal; and d) determining a level of expression of at least one gene identified in Tables 1-15 in brain cells of the cocaine-addicted subject. The level of expression is compared to that observed in brain cells of a cocaine-addicted subject to which the candidate agent is not administered, wherein a change in expression level is indicative of the candidate having efficacy in preventing or inhibiting cocaine addiction-related behavior.

[22] Still other embodiments involve: c) administering the candidate agent identified in b) to a cocaine-addicted subject that is undergoing withdrawal; and d) determining whether the withdrawal symptoms exhibited by the subject are reduced upon administration of the candidate agent.

[23] Also provided by the invention are methods for identifying an agent to be tested for an ability to prevent or inhibit addiction related behavior. These methods involve: a) exposing a cocaine-addicted subject or brain cells of a cocaine-addicted subject

to a candidate agent, wherein the cocaine-addicted subject is undergoing withdrawal; b) determining a level of expression of at least one gene in the cocaine-addicted subject or brain cells of the cocaine-addicted subject, wherein the at least one gene is identified in Tables 1-15; and c) comparing the level of expression of the gene in the cocaine-addicted subject or brain cells of the cocaine-addicted subject in the presence of the candidate agent with the level of expression of the gene in the cocaine-addicted subject or brain cells of the cocaine-addicted subject in the absence of the candidate agent. A reversal in the level of expression of the gene in cocaine-addicted subject or brain cells of the cocaine addicted subject in the presence of the candidate agent relative to the level of expression of the gene in the absence of the candidate agent indicates that the candidate agent is an agent to be tested for the ability to prevent or inhibit addiction related behavior.

[24] The invention also provides methods for identifying an agent to be tested for an ability to prevent or inhibit cocaine addiction-related behavior. These methods involve:

a) contacting a brain tissue sample from each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

b) detecting a level of expression of at least one gene in both tissue samples, wherein the gene encodes a polypeptide selected from the group consisting of hypertension-regulated vascular factor, myelin-associated basic protein, PB cadherin, calcitonin receptor, melanocortin 4 receptor, ALK-7 kinase and retroposon.

c) subtracting the level of expression of the gene in the sample obtained from the cocaine addiction-free subject from the level of expression of the gene in the sample obtained from the subject having cocaine addiction-related behavior to provide a first value;

d) administering a candidate agent to each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

e) detecting a level of expression of at least one gene in both tissue samples obtained from the subjects treated with the candidate agent;

f) subtracting the level of expression of the at least one gene in the sample obtained from the treated cocaine addiction-free subject from the level of expression of the gene in the sample obtained from the treated subject having the cocaine addiction-related behavior to provide a second value; and

g) comparing the second value with the first value wherein a decreased second value relative to the first value is indicative of an agent to be tested for an ability to prevent or inhibit cocaine addiction-related behavior.

[25] In some embodiments, the invention provides methods for identifying agents to be tested for an ability to prevent or inhibit cocaine addiction-related behavior that involve:

a) obtaining a brain tissue sample from each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

b) detecting a level of expression of at least one gene in both tissue samples, wherein the gene encodes a polypeptide selected from the group consisting of GABA-B receptor subunit gb2, cell adhesion-like molecule, bos taurus-like neuronal axonal protein, similar to mouse chemokine-like factor, FRA-2, a polypeptide similar to human oxygen-regulated protein, a polypeptide similar to mouse mrg1 protein, pentraxin, malic enzyme, olfactomedin-related protein, arc-growth factor enriched in dendrites, protein tyrosine phosphatase, krox, neuritin, microtubule-associated protein 2d and CB1 cannabinoid receptor;

c) subtracting the level of expression of the gene in the sample obtained from the cocaine addiction-free subject from the level of expression of the gene of the sample obtained from the subject having cocaine addiction-related behavior to provide a first value;

d) administering a candidate agent to each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

e) detecting a level of expression of the gene in both tissue samples obtained from the subjects treated with the candidate agent;

f) subtracting the level of expression of the gene in the sample obtained from the treated cocaine addiction-free subject from the level of expression of the gene in the sample obtained from the treated subject having the cocaine addiction-related behavior to provide a second value; and

g) comparing the second value with the first value wherein an increased second value relative to the first value is indicative of an agent to be tested for an abilty to prevent or inhibit cocaine addiction related behavior.

BRIEF DESCRIPTION OF THE DRAWINGS

[26] **Figure 1.** Diagrammatic representation of the primary pathways through which stress, drugs of abuse and drug-associated conditioned stimuli are hypothesized to trigger drug craving and relapse to drug-seeking. Stress and conditioned stimuli can activate excitatory glutamatergic projections to the VTA from the PfC, amygdala (Amyg) and hippocampus (Hipp), while priming injections of drugs directly stimulate dopamine (DA) release in the NAc. In this sense, dopamine release in the NAc may be a may be a final common trigger of drug craving by all three stimuli. At the level of NAc neurons, dopamine from the VTA modulates direct excitatory signals from the PfC, Amyg and Hipp where complex spatio-temporal integration of relapse-related information occurs. Studies showing involvement of these brain regions in reinstatement of drug-seeking suggest that long-term changes in gene expression in these regions would alter the functionality of this circuitry, and could produce profound changes in reactivity to stimuli that trigger drug craving and relapse to drug-seeking (adapted from Self et al., *supra*).

[27] **Figure 2.** Time-dependent increases in drug-seeking behavior during forced abstinence from cocaine self-administration. Groups of rats (ns=8-25) were balanced such that each group averaged similar levels of cocaine intake on the last 3 days of self-administration testing (1.0 mg/kg/infusion in 4hour test sessions during the dark cycle). Following different periods of forced abstinence rats were returned to the drug-paired environment, and non-reinforced responding at the drug-paired lever was measured during 6 daily 4-hour extinction tests. Rats tested during the third and sixth week of forced abstinence showed significantly greater levels of drug-seeking behavior during the first 2 extinction tests than rats tested during the first week of forced abstinence (*$P < 0.05$; Fisher's LSD).

[28] **Figure 3.** The Cocaine Abstinence Effect is evident at both the beginning (A) and end (B) of extinction testing. Selective responding at the drug-paired, rather than inactive, lever reflects the level of effort exerted by animals to self-administer cocaine (i.e., drug-seeking behavior). The left panel (A) depicts non-reinforced responding during the first hour of the initial 4-hour extinction test in groups of animals with forced abstinence ranging from 1 day to 5 weeks. The initial level of spontaneous drug-seeking behavior more than tripled at 2 and 5 weeks of forced abstinence when compared to rats tested after 1 day of forced abstinence (***$P \leq 0.001$). Following extinction testing, the

ability of cues associated with cocaine infusions (house light off; lever light on; pump noise, vehicle infusion) to induce relapse to drug-seeking behavior was measured (B). The cues were non-contingently delivered for 10 seconds every 2 minutes for 1 hour immediately following the final extinction test. The level of drug-seeking behavior during cue-induced relapse doubled at 6 weeks when compared to 1 week of forced abstinence (**$P<0.01$; Fisher's LSD; 3-4 non-responders/group were not included in relapse analysis). Note that baseline response rates in the 1-hour period preceding cue exposure were similar for all 3 groups of rats (mean group responses ranged from 5.4-9.2).

[29] **Figure 4.** Effects of extinction training on withdrawal-induced changes in gene expression following 1 week abstinence from 12 days (4 hours/day at 1.0 mg/kg/injection) of cocaine self-administration. Example GeneChip profiles of mRNAs from NAc shell tissue are shown for 2 genes differentially regulated during early withdrawal by extinction training. Expression of the retroviral derived rat brain retroposon gene is elevated 88% during withdrawal from cocaine self-administration, but decreased 49% in animals that underwent 4 hours/day of extinction training, when compared to control values (see Table 1). The CB1 cannabinoid receptor is reduced 53% during withdrawal from cocaine self-administration, but is normalized (19% increase relative to control values) in animals that experienced extinction training during withdrawal. The top row of highlighted boxes in each array contains several different oligonucleotide sequences (25 bases/each) spanning the target sequence, while the bottom row contains a 1 base mismatch in the same sequences.

[30] **Figure 5.** Time-course and overall experimental strategy to identify changes in gene expression produced by cocaine self-administration (SA) abstinence and extinction. Arrows denote the time of sacrifice and dissection of the NAc shell for analysis with gene expression profiling. Group I remained in their home cages during 1 week of abstinence. Groups II and IV underwent 1 week of extinction training 1 week prior to sacrifice. Not shown are three groups that simultaneously underwent saline self-administration and were sacrificed along with Groups I, II and IV.

[31] **Figure 6.** Diagrammatic representation of tissue punches of limbic brain regions collected from animals during 1 week abstinence from cocaine self-administration for oligonucleotide array analysis. A "half-moon" outer punch of NAc shell was collected with a 12-gauge tissue punch. Each punch was taken from chilled brain slices

immediately following sacrifice. The anatomical plates illustrate the posterior side of each 1.2-1.5 mm thick brain slice. For the current study, only the NAc shell was used. Other brain regions shown were also dissected but will be used for later studies.

[32] **Figure 7.** GABA-B receptor subunit gb2 protein levels are increased by extinction training in the NAc shell as measured by Western Blot. Values are expressed as a percentage of the mean of the control group.

[33] **Figures 8-10.** Cannabinoid receptor CB1 protein levels are increased by cocaine withdrawal in the NAc shell as measured by Western Blot. Three different bands specific for CB1 were detected and quantitated separately: Figure 8, 70 kDa glycosylated species; Figure 9, upper 50 kDa nonglycosylated species; and Figure 10, lower 50 kDAa glycosylated species.

DETAILED DESCRIPTION OF THE INVENTION

[34] All patent applications, patents and literature references cited herein are hereby incorporated by reference in their entirety.

[35] The present invention relates to the identification of genes that are up- or down- regulated in particular regions of the brain of rats undergoing cocaine withdrawal compared with rats that are free from cocaine addiction (control) as shown below (see Tables 1-16.

[36] As used herein, the term "up-regulated" with respect to these genes means that the expression of these genes is higher in rats undergoing cocaine withdrawal compared with rats that are free from cocaine addition. Such up-regulation refers to at least about a two-fold change.

[37] As used herein, the term "down-regulated" with respect to these genes means that the expression of these genes is lower in cocaine-addicted rats undergoing withdrawal compared with rats that are free from cocaine addiction. Such down-regulation refers to at least about a two-fold changed.

[38] Importantly, as shown in Table 1 the up- or down-regulation of many of these genes observed in the brain tissue of cocaine-addicted rats undergoing withdrawal is reversed upon subjecting these rats to extinction training. These results indicate a causal relationship between extinction-induced neuroadaptations in these genes and the propensity for behavior associated with cocaine addiction, particularly cocaine-seeking behavior.

Accordingly, these differentially expressed genes can form the basis for novel agents useful in the treatment of cocaine addiction and in reducing, inhibiting or preventing addiction-related behavior in individuals suffering from cocaine addiction. In addition, these differentially expressed genes can be utilized to identify agents that inhibit or prevent behavior associated with cocaine addiction. Gene expression is typically assessed about 1-2 weeks after withdrawal.

[39] The complete sequences of the genes listed in Tables 1-15 are available from GenBank database using the assigned accession numbers (as in Table 1) or part of the probe set identification numbers which indicate the accession numbers of the genes. For example, Probe set identification number "rc_AA875032_at listed in Table 3 corresponds to GenBank Accession No. AA875032. The sequences of these genes in GenBank, and their probe identification and accession numbers are expressly incorporated herein by reference.

[40] The brain regions where these genes are differentially expressed include the nucleus accumbens shell (Nac shell), the nucleus accumbens core (Nac core), the central nucleus of the amygdala (CeA), the ventral tegmental area (VTA) and the medial prefrontal cortex (mPFC). Evidence linking behavior associated with cocaine addiction to the aforementioned brain regions further support the involvement of the aforementioned genes expressed in these brain regions in such behavior. As stated above, although it has not been clearly resolved, cue- and stress-induced reinstatement of drug-seeking behavior may involve both dopamine-dependent and dopamine-independent neural substrates (reviewed by Self et al., *supra*). However, the basolateral amygdala (BLA), as well the CeA and related extended amygdala structures recently have been implicated in cue-and stress-induced reinstatement of drug-seeking behavior (see Meil et al, "Lesions of the Basolateral Amygdala a Bolish of the Ability of Drug Associated Cues to Reinstate Responding During Withdrawal from Self-Administered Cocaine", Behav., Brain Res., Vol. 87, pp. 139-148 (1997); and Erb et al., "A Role for the Bed Nucleus of the Stria Terminalis, but Not the Amygdala, in the Effects of Corticotroopin-Releasing Factor on Stress-Induced Reinstatement of Cocaine Seeking", J. Neurosci., Vol. 19, pp. C1-C6 (1999)). The CeA sends a direct excitatory projection to VTA neurons (see Gonzales et al., "Amydalonigral Pathway: An Anterograde Study in the Rat with Phaseolus Vulgaris Leucoagglutinin", J. Comp. Neurol., Vol. 297, pp. 182-200 (1990); and Wallace et al., "Organization of Amygdaloid Projections to Brainstem Dopaminergic, Noradrenergic, and Adrenergic Cell

Groups in the Rat", Brain Res. Bull., Vol. 28, pp. 447-454 (1992)), which could mediate dopamine release in response to cues and stress. Other brain regions involved in relapse may include the PfC, where excitatory projections to dopamine neurons in the VTA activate dopamine release in the NAc (see Moghaddam, "Stress Preferentially Increases Extraneuronal Levels of Excitatory Amino Acids in the Prefrontal Cortex: Comparison to Hippocampus and Basal Ganglia", J. Neurochem., Vol. 60, pp. 1650-1657 (1993); Taber, Das and Fibiger, "Cortical Regulation of Subcortical Dopamine Release: Mediation Via the Ventral Tegmental Area", J. Neurochem., Vol. 65, pp. 1407-1410 (1995); and Karreman et al., "The Prefrontal Cortex Regulates the Basal Release of Dopamine in the Limbic Striatum: An Effect Mediated by Ventral Tegmental Area", J. Neurochem., Vol. 66, pp. 589-598 (1996)). Similarly, recent studies have found that electrical stimulation of hippocampal-subiculuar outputs elevates dopamine levels in the NAc via excitatory inputs to the VTA (see Legault et al., "Chemical Stimulation of the Ventral Hippocampus Elevates Nucleus Accumbens Dopamine by Activating Dopaminergic Neurons of the Ventral Tegmental Area", J. Neurosci., Vol. 20, pp. 1635-1642 (2000)), and also reinstates cocaine-seeking behavior (see Vorel et al., "Electrical Stimulation of Ventral Subiculum Induced Relapse to Cocaine Self-Administration", Soc. Neurosci. Abstr., p. 2170 (1998). Another area of excitatory convergence is the NAc, where excitatory inputs from these the PfC, BlA and subiculum innervate medium spiny neurons receiving dopamine inputs from the VTA. Excitatory neurotransmission in the NAc also has been implicated in reinstatement of cocaine-seeking behavior (see Cornish et al., *supra*). Together, these brain regions all form a complex circuit with primary sites of convergence in both the VTA and NAc of the mesolimbic dopamine system, as depicted in Figure 1.

[41] Any selection of at least one of the genes listed in Tables 1-15 can be utilized as a therapeutic target for inhibiting or preventing behavior associated with cocaine addiction. Preferably at least one of the genes is identified in Tables 1, 5, 8, 11 and 14, and more preferably at least one gene is identified in Table 1. In particularly useful embodiments, a plurality of these genes, i.e. two or more, can be selected and their expression monitored simultaneously to provide expression profiles for use in various aspects. For example, expression profiles of these genes can provide valuable molecular tools for rapidly identifying agents that alter these expression profiles. Particularly preferred genes from Tables 1-15 that are useful as therapeutic targets include those listed in Table 16.

[42] In one aspect, methods of treating addiction-related behavior in a subject, e.g., a human or animal, suffering from cocaine addiction are provided which involve preventing or inhibiting cocaine-addiction related behavior utilizing various therapeutics that modulate the transcription/translation of these differentially expressed genes or that modulate the activity of proteins encoded by these genes. As used herein, cocaine refers to cocaine itself and derivatives of cocaine, e.g., crack. As used herein the term "addiction-related behavior" refers to behavior resulting from cocaine use and is characterized by apparent total dependency on cocaine. Symptomatic of such behavior is (i) overwhelming involvement with the use of cocaine; (ii) the securing of its supply; and (iii) a high probability of relapse following withdrawal. For example, in cocaine users addiction-related behavior typically includes behavior associated with three stages of drug effects. In the first stage, acute intoxication, "binge", is euphoric, marked by decreased anxiety, enhanced self-confidence and sexual appetite. In the second stage, the "crash" replaces the euphoric feeling with anxiety, fatigue, irritability and depression. The third stage, "anhedonia" is a time of limited ability to experience pleasure from normal activities and of craving for the euphoric effects of cocaine. In particularly useful embodiments, the cocaine-addiction related behavior is cocaine seeking. As used herein, cocaine seeking which is a behavior measured in cocaine-addicted animals such as rats is assumed to be analogous to the behavior, cocaine craving, that is observed in humans.

[43] Examples of suitable therapeutic agents for inhibiting or preventing cocaine addiction- related behavior include, but are not limited to, antisense sequences, ribozymes, double-stranded RNAs, small inhibitory RNA (siRNA), agonists and antagonists as described in detail below.

[44] As used herein, the term "antisense" refers to nucleotide sequences that are complementary to a portion of an RNA expression product of at least one of the disclosed genes. "Complementary" nucleotide sequences refer to nucleotide sequences that are capable of base-pairing according to the standard Watson-Crick complementarity rules. That is, purines will base- pair with pyrimidine to form combinations of guanine:cytosine and adenine:thymine in the case of DNA, or adenine:uracil in the case of RNA. Other less common bases, e.g., inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others may be included in the hybridizing sequences and will not interfere with pairing.

[45] When introduced into a host cell, antisense nucleotide sequences specifically hybridize with the cellular mRNA and/or genomic DNA corresponding to the gene(s) so as to inhibit expression of the encoded protein, e.g., by inhibiting transcription and/or translation within the cell.

[46] The isolated nucleic acid molecule comprising the antisense nucleotide sequence can be delivered, e.g., as an expression vector, which when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the encoded mRNA of the gene(s). Alternatively, the isolated nucleic acid molecule comprising the antisense nucleotide sequence is an oligonucleotide probe which is prepared *ex vivo* and, which, when introduced into the cell, results in inhibiting expression of the encoded protein by hybridizing with the mRNA and/or genomic sequences of the gene(s).

[47] The oligonucleotide can include artificial internucleotide linkages which render the antisense molecule resistant to exonucleases and endonucleases, and thus are stable in the cell. Examples of modified nucleic acid molecules for use as antisense nucleotide sequences are phosphoramidate, phosporothioate and methylphosphonate analogs of DNA as described, e.g., in U.S. Patent No. 5,176,996; 5,264,564; and 5,256,775. General approaches to preparing oligomers useful in antisense therapy are described, e.g., in Van der Krol., BioTechniques 6:958-976, 1988; and Stein et al., Cancer Res. 48:2659-2668, 1988.

[48] Typical antisense approaches, involve the preparation of oligonucleotides, either DNA or RNA, that are complementary to the encoded mRNA of the gene. The antisense oligonucleotides will hybridize to the encoded mRNA of the gene and prevent translation. The capacity of the antisense nucleotide sequence to hybridize with the desired gene will depend on the degree of complementarity and the length of the antisense nucleotide sequence. Typically, as the length of the hybridizing nucleic acid increases, the more base mismatches with an RNA it may contain and still form a stable duplex or triplex. One skilled in the art can determine a tolerable degree of mismatch by use of conventional procedures to determine the melting point of the hybridized complexes.

[49] Antisense oligonucleotides are preferably designed to be complementary to the 5' end of the mRNA, e.g., the 5'untranslated sequence up to and including the regions complementary to the mRNA initiation site, i.e., AUG. However, oligonucleotide sequences that are complementary to the 3' untranslated sequence of mRNA have also been shown to be effective at inhibiting translation of mRNAs as described e.g., in

Wagner, Nature 372:333, 1994. While antisense oligonucleotides can be designed to be complementary to the mRNA coding regions, such oligonucleotides are less efficient inhibitors of translation.

[50] Regardless of the mRNA region to which they hybridize, antisense oligonucleotides are generally from about 15 to about 25 nucleotides in length.

[51] The antisense nucleotide can also comprise at least one modified base moiety, e.g., 3-methylcytosine, 5,-methylcytosine, 7-methylguanine, 5-fluorouracil, 5-bromouracil, and may also comprise at least one modified sugar moiety, e.g., rabinose, hexose, 2-fluorarabinose, and xylulose.

[52] In another embodiment, the antisense nucleotide sequence is an alpha-anomeric nucleotide sequence. An alpha-anomeric nucleotide sequence forms specific double stranded hybrids with complementary RNA, in which, contrary to the usual beta-units, the strands run parallel to each other as described e.g., in Gautier et al., Nucl. Acids. Res. 15:6625-6641, 1987.

[53] Antisense nucleotides can be delivered to cells which express the described genes *in vivo* by various techniques, e.g., injection directly into the prostate tissue site, entrapping the antisense nucleotide in a liposome, by administering modified antisense nucleotides which are targeted to the prostate cells by linking the antisense nucleotides to peptides or antibodies that specifically bind receptors or antigens expressed on the cell surface.

[54] However, with the above-mentioned delivery methods, it may be difficult to attain intracellular concentrations sufficient to inhibit translation of endogenous mRNA. Accordingly, in a preferred embodiment, the nucleic acid comprising an antisense nucleotide sequence is placed under the transcriptional control of a promoter, i.e., a DNA sequence which is required to initiate transcription of the specific genes, to form an expression construct. The use of such a construct to transfect cells results in the transcription of sufficient amounts of single stranded RNAs to hybridize with the endogenous mRNAs of the described genes, thereby inhibiting translation of the encoded mRNA of the gene. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of the antisense nucleotide sequence. Such vectors can be constructed by standard recombinant technology methods. Typical expression vectors include bacterial plasmids or phage, such as those of the pUC or Bluescript.TM plasmid

series, or viral vectors such as adenovirus, adeno-associated virus, herpes virus, vaccinia virus and retrovirus adapted for use in eukaryotic cells. Expression of the antisense nucleotide sequence can be achieved by any promoter known in the art to act in mammalian cells. Examples of such promoters include, but are not limited to, the promoter contained in the 3' long terminal repeat of Rous sarcoma virsu as described, e.g., in Yamamoto et al., Cell 22: 787-797, 1980; the herpes thymidine kinase promoter as described e.g., in Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445, 1981; the SV40 early promoter region as described e.g., in Bernoist and Chambon, Nature 290:304-310, 1981; and the regulatory sequences of the metallothionein gene as described, e.g., in Brinster et al., Nature 296:39-42, 1982.

[55] Ribozymes are RNA molecules that specifically cleave other single-stranded RNA in a manner similar to DNA restriction endonucleases. By modifying the nucleotide sequences encoding the RNAs, ribozymes can be synthesized to recognize specific nucleotide sequences in a molecule and cleave it as described, e.g., in Cech, J. Amer. Med. Assn. 260:3030, 1988. Accordingly, only mRNAs with specific sequences are cleaved and inactivated.

[56] Two basic types of ribozymes include the "hammerhead"-type as described for example in Rossie et al. Pharmac. Ther. 50:245-254, 1991; and the hairpin ribozyme as described, e.g., in Hampel et al, Nucl. Acids Res. 18:299-304, 1999 and U.S. Patent No. 5,254,678. Intracellular expression of hammerhead and hairpin ribozymes targeted to mRNA corresponding to at least one of the disclosed genes can be utilized to inhibit protein encoded by the gene.

[57] Ribozymes can either be delivered directly to cells, in the form of RNA oligonucleotides incorporating ribozyme sequences, or introduced into the cell as an expression vector encoding the desired ribozymal RNA. Ribozyme sequences can be modified in essentially the same manner as described for antisense nucleotides, e.g., the ribozyme sequence can comprise a modified base moiety.

[58] Double-stranded RNA, i.e., sense-antisense RNA, corresponding to at least one of the disclosed genes, can also be utilized to interfere with expression of at least one of the disclosed genes. Interference with the function and expression of endogenous genes by double-stranded RNA has been shown in various organisms such as *C. elegans* as described, e.g., in Fire et al., Nature 391:806-811, 1998; drosophilia as described, e.g., in

Kennerdell et al., Cell 95(7):1017-26, 1998; and mouse embryos as described, e.g., in Wianni et al., Nat. Cell Biol. 2(2):70-5, 2000. Such double-stranded RNA can be synthesized by *in vitro* transcription of single-stranded RNA read from both directions of a template and *in vitro* annealing of sense and antisense RNA strands. Double-stranded RNA can also be synthesized from a cDNA vector construct in which the gene of interest is cloned in opposing orientations separated by an inverted repeat. Following cell transfection, the RNA is transcribed and the complementary strands reanneal. Double-stranded RNA corresponding to at least one of the disclosed genes could be introduced into a prostate cell by cell transfection of a construct such as that described above.

[59] The term "antagonist" refers to a molecule which when bound to the protein encoded by the gene inhibits its activity. Antagonists can include, but are not limited to, peptides, proteins, carbohydrates and small molecules. In a particularly useful embodiment, the antagonist is an antibody specific for the protein expressed by the at least one gene.

[60] The term "agonist" as used herein refers to any natural or synthetic molecule which, when bound to the expressed protein, increases or prolong the duration of the effect of the protein. Agonists can include proteins, nucleic acids, carbohydrates or any other molecules that bind to and modulate the effect of the protein.

[61] In one embodiment, a method of inhibiting addiction-related behavior in a subject suffering from cocaine addiction is provided which comprises administering to the subject a therapeutically effective amount of a therapeutic agent which has the ability to modulate the transcription/translation of at least one gene or the activity of a protein encoded by the genes, wherein the at least one gene is identified in Tables 1, 2 and 4-15. In the case where the therapeutic agent is an antisense sequence, an isolated nucleic acid molecule encoding a ribozyme, or a double stranded RNA, such an agent modulates the transcription/translation of the gene. In the case wherein the therapeutic agent is an antagonist or agonist, such an agent modulates the activity of a protein encoded by the gene.

[62] As used herein, the term "isolated" nucleic acid molecule means that the nucleic acid molecule is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturallly occurring nucleic acid molecule is not isolated, but the same nucleic acid molecule, separated from some or all of the coexisting materials in the natural system, is isolated, even if subsequently reintroduced

into the natural system. Such nucleic acid molecules could be part of a vector or part of a composition and still be isolated, in that such vector or composition is not part of its natural environment.

[63] As used herein, the term "modulate" with respect to transcription/translation refers to the up-or down-regulation of transcription/translation of the gene, i.e., that is "modulate" includes either an increase or a decrease in expression of the at least one gene. The direction of modulation affected by the therapeutic agent depends on which gene is being modulated. For example, the calcitonin receptor gene is upregulated in the Nac Shell of cocaine-addicted rats during cocaine withdrawal. Accordingly, an antisense sequence, a ribozyme, or a double stranded RNA modulates expression of the calcitonin gene by blocking the "up-regulation" of expression of this gene or reversing or "down-regulating" the expression of this gene.

[64] As used herein, the term "modulate" with respect to activity of a protein encoded by the gene, refers to an alteration, i.e., increase or decrease, in the activity of a protein encoded by the gene. For example, the gene encoding malic enzyme is down-regulated in Nac Shell of cocaine-addicted rats during cocaine withdrawal. Accordingly, an agonist that would increase the activity of the malic enzyme can aid in inhibiting addiction-related behavior.

[65] In a preferred embodiment of the method for inhibiting or preventing cocaine-addiction related behavior, the at least one gene identified in Table 1 encodes a polypeptide selected from the group consisting of GABA-B receptor subunit gb2, myelin-associated basic protein, calcitonin receptor, Bos taurus-like neuronal axonal protein, FRA-2, a polypeptide similar to human oxygen-regulated protein, a polypeptide similar to mouse mrg 1 protein, pentraxin, olfactomedin-related protein, arc-growth factor (enriched in dendrites), protein tyrosine phosphatase, melanocortin 4 receptor, ALK-7 kinase, neuritin and CB1 cannabinoid receptor. More preferably, the at least one gene identified in Table 1 encodes GABA-B receptor subunit gb2, FRA-2 and CB1 cannabinoid receptor. In some embodiments of the method for inhibiting or preventing cocaine addiction-related behavior, the at least one gene identified in Table 1 does not encode melanocortin 4 receptor.

[66] In another preferred embodiment of the method for inhibiting or preventing cocaine addiction-related behavior, the at least one gene is identified in Table 2.

[67] In another preferred embodiment of the above method, the at least one gene is identified in Table 4, and more preferably encodes a polypeptide selected from the group consisting of GABAB receptor 1d, tyrosine kinase receptor RET and Neurodap-1.

[68] In another preferred embodiment of this method, the at least one gene is identified in Table 5, and more preferably encodes a polypeptide selected from the group consisting of inhibin alpha-subunit and vesicular transport factor.

[69] In another preferred embodiment of this method, the at least one gene is identified in Table 6, and more preferably encodes a polypeptide selected from the group consisting of GABAB receptor 1c and phosphatidylinositol 4-kinase.

[70] In another preferred embodiment of this method, the at least one gene is identified in Table 7 and more preferably encodes a polypeptide selected from the group consisting of somatostain-14 and kainate receptor submit (ka2).

[71] In another preferred embodiment of this method, the at least one gene is identified in Table 8, and more preferably encodes a polypeptide selected from the group consisting of melanocortin-3 receptor, somatostatin, metabotropic glutamate receptor 3, NCAM polypeptide and synaptic SAPAP-interacting protein.

[72] In another preferred embodiment of this method, the at least one gene is identified in Table 9, and more preferably encodes calpastatin.

[73] In another preferred embodiment of this method, the at least one gene is identified in Table 10, and more preferably encodes a polypeptide selected from the group consisting of RAC protein kinase alpha, alpha-2B-adrenergic receptor and SNAP-25A.

[74] In another preferred embodiment of this method, the at least one gene is identified in Table 11, and more preferably encodes a polypeptide selected from the group consisting of oxytosin/neurophysin, NMDAR2C and GABA-A receptor epsilon.

[75] In another preferred embodiment of thiis method, the at least one gene is identified in Table 12, and preferably encodes a polypeptide selected from the group consisting of phosphodiesterase I, tyrosine phosphatase and dopamine transporter.

[76] In yet another preferred embodiment of this method, the at least one gene is identified in Table 13, and preferably encodes synaptotagmin IV homolog.

[77] In another useful embodiment of this method, the at least one gene is identified in Table 14, and preferably encodes a polypeptide selected from the group consisting of calmodulin, protein kinase rMNK2, phospholipase C-beta1b.

[78] In another useful embodiment of this method, the at least one gene is identified in Table 15, and preferably encodes a polypeptide selected from the group consisting of phosphatidylinositol 4-kinase and protein-tyrosine-phosphatase.

[79] A "therapeutically effective amount" of a therapeutic agent refers to a sufficient amount of the therapeutic agent to prevent or inhibit cocaine addiction-related behavior in a subject suffering from cocaine addiction. The determination of a therapeutically effective amount is well within the capability of those skilled in the art. For any therapeutic, the therapeutically effective dose can be estimated in animal models, usually mice, rats, rabbits, dogs or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

[80] Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in experimental animals, e.g., ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}. Antisense nucleotides, ribozymes, double-stranded RNAs, antagonists and agonists, and other therapeutic agents that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the subject and the route of administration.

[81] The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy.

[82] Normal dosage amounts may vary from 0.1-100,000 mg, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to

practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for antagonists.

[83] For therapeutic applications, the therapeutic agents are preferably administered as pharmaceutical compositions containing the therapeutic agent in combination with one or more pharmaceutically acceptable carriers. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose and water. The compositions may be administered to a subject, or in combination with other agents or drugs.

[84] In another aspect, the present invention provides screening methods for identifying agents to be tested for the ability to inhibit or prevent cocaine addiction-related behavior. The screening methods are typically designed to find candidate agents that can interact, i.e., bind, to proteins encoded by these differentially expressed genes, and then these agents can be used in assays that ascertain the ability of the candidate agent to modify the activity of the protein. Such binding and activity assays can be performed in cell-free systems, e.g., in a reconstituted protein mixture or a cell membrane preparation, and in cells, particularly recombinant cells expressing the protein encoded by the gene. In particularly useful embodiments of these screening methods, candidate agents are screened in animal models for their ability to reverse, i.e., either increase or decrease, the expression of at least one of the disclosed genes that are upregulated or down regulated by cocaine withdrawal.

[85] As used herein, the term "candidate agent" refers to any molecule that is capable of interacting, i.e., binding to, and/or increasing or decreasing the activity of, a protein encoded by one of the disclosed genes. The candidate agent can modify the structure of the encoded protein to thereby alter the activity of the protein. The candidate agent also refers to any molecule that is capable of increasing/decreasing the level of mRNA corresponding to or protein encoded by at least one of the disclosed genes. The candidate agent can be natural or synthetic molecules such as proteins or fragments thereof, antibodies, nucleic acid molecules, e.g., antisense nucleotides, ribozymes, double-stranded RNAs, organic and inorganic compounds and the like.

[86] In one embodiment, cell-free assays for identifying such candidate agents comprise combining in a reaction mixture, i.e, a cell-free system or cell-based system, a candidate agent with a protein encoded by one of the disclosed genes in Tables 1-15 and

determining the interaction, i.e., binding, of the candidate agent to the protein or modulation of the activity of the protein. In other embodiments, a fragment of the protein encoded by the disclosed gene can be combined with the candidate agent. Preferred proteins include those encoded by genes identified in Tables 1, 5, 8, 11 and 14. More preferred proteins are those encoded by the preferred listed genes for each of Tables 1, 2, and 4-15, and preferably Table 1 as described above in the methods for inhibiting addiction-related behavior. In some embodiments of this cell-free assay, the gene identified in Table 1 does not encode CB1 cannibinoid receptor.

[87] In a particularly useful embodiment, the protein encoded by the disclosed gene or the candidate agent is immobilized to an insoluble support to facilitate separation of complexes of the protein/candidate agent from uncomplexed forms of the protein and automation of the assay. The insoluble support may be solid or porous and possess any shape. Examples of suitable solid supports include, but are not limited to, microtitre plates and arrays, micro-centrifuge tubes, test tubes, membranes and beads. Particularly useful methods of binding include, but are not limited to, the use of antibodies, direct binding to ionic supports, and chemical crosslinking. Subsequent to binding of the protein or agent to the support, unbound material is removed by washing.

[88] In a preferred embodiment, the protein encoded by the gene is bound to the insoluble support, and the candidate agent is then added. Alternatively, the candidate agent is bound to the solid support and the protein encoded by the gene is added.

[89] Determination of the binding of the candidate agent to the encoded protein can be carried out by standard methods. For example, the candidate agent can be labeled, and binding determined by, e.g, attaching the protein or fragment thereof to the insoluble support, adding the labeled candidate agent, washing off unbound candidate agent, and determinng whether any label is bound to the support.

[90] The term "labeled" means that the candidate agent or protein is either directly or indirectly labeled with a label to provide a detectable signal, e.g., enzymes, antibodies, radioisotopes, fluorescers, chemiluminescers, or specific binding molecule pairs such as biotin and streptavidin. For example, the protein can be biotinylated using biotin NHS (N-hydroxysuccinimide), using well-known techniques and immobilized in the well of streptavidin-coated plates.

[91] Interaction (binding) between molecules can also be assessed by using real-time BIA (Biomolecular Interaction Analysis, Pharmacia Biosensor AB), which detects surface plasmon resonance, an optical phenomenon. Detection depends on changes in the mass concentration of mass macromolecules at the biospecific interface and does not require labeling of the molecules. In one useful embodiment, a library of candidate agents, such as organic compounds, can be immobilized on a sensor surface, e.g., a wall of a micro-flow cell. A solution containing the protein or functional fragment thereof is then continuously circulated over the sensor surface. An alteration in the resonance angle, as indicated on a signal recording, indicates the occurrence of an interaction. This technique is described in more detail in BIAtechnology Handbook by Pharmacia.

[92] In another embodiment, the binding of the candidate agent to the protein encoded by the gene can be determined using competitive binding assays wherein a competitor, i.e., a substance known to bind to the encoded protein such as an antibody, ligand, peptide, etc., is combined with the encoded protein, either prior to or subsequent to combining the protein with the candidate agent. For example, the competitor can be added to the protein followed by the candidate agent. Displacement of the competitor indicates that the candidate agent is binding to the encoded protein. In this embodiment, the candidate agent or competitor can be labeled. Accordingly, if a labeled competitor is used, the presence of the label in the wash removed from the insoluble support, indicates displacement by the candidate agent. Alternatively, if the candidate agent is labeled, the presence of the label on the insoluble support indicates displacement of the competitor.

[93] Cell-free assays can also be used to identify agents which interact with a protein encoded by one of the disclosed genes and modulate the activity of this protein. In one embodiment, the protein encoded by one of the disclosed genes is incubated with a candidate agent, such as an organic compound and the catalytic activity of the protein is determined.

[94] In another aspect, a cell-based assay is provided for screening candidate agents that bind to a protein encoded by one of the disclosed genes. The method comprises providing a recombinant cell expressing a protein encoded by one of the genes identified in Tables 1-15, contacting the cell with a candidate agent; and determining the binding of the candidate agent to the protein. As used herein, the term "recombinant cell" refers to a cell that has been transfected by one of the disclosed genes, wherein the cell expresses the gene.

The recombinant cell is preferably a mammalian cell, an insect cell, a xenopus cell or an oocyte. Cells used as controls include cells that are substantially identical to the recombinant cells, but do not express the proteins encoded by the disclosed genes. The binding of the candidate agent to the protein expressed by the cell can be determined by e.g., detecting a signal in the cell, e.g., alterations in second messengers which are sensitive to binding of the candidate agent. Such a recombinant cell further comprises a reporter gene operatively linked to a transcriptional control sequence which is responsive to an intracellular signal, i.e., a second messenger, transduced by interaction of the candidate agent with the protein expressed by the recombinant cell. For example, cyclic AMP accumulation induced by CB1 activation can be measured using a cyclic AMP response element (CRE) reporter assay. Candidate agents that enhance or suppress expression of the reporter interact with either CB1 or its signal transduction system.

[95] The term "transcriptional control sequence" refers to DNA sequences, such as initiator sequences, enhancer sequences and promoter sequences, which induce, repress or ortherwise control the transcription of protein encoding nucleic acid sequence to which they are operatively linked. Upon induction of the transcriptional control sequence by the second messenger, the reporter gene is expressed thereby providing a quantifiable and detectable signal, e.g., color, fluorescence, luminescence, cell growth, drug resistance, etc., that determines binding of the candidate agent to the protein. Examples of such reporter genes include, but are not limited to, luciferase, alkaline phosphatase, chloramphenicol acetyl transferase and betagalactosidase. In some embodiments, the protein encoded by one of the genes identified in Table 1 is not CB1 cannabinoid receptor. In some embodiments, modulation of binding of the protein encoded by one of the disclosed genes to the candidate agent can be determined in the presence of a target protein or target peptide which is known to bind to the a protein encoded by one of the disclosed genes.

[96] In yet another embodiment, the effect of a candidate agent on the transcription of one of the genes disclosed in Tables 1-15 is determined by transfection experiments using a reporter gene operatively linked to at least a portion of a transcriptional control sequence of a gene identified in Tables 1-15.

[97] Assays based on animal models or cells obtained from such animals can also be used to identify agents which modulate the expression of a gene identified in Tables 1-15, that has undergone up- or down-regulation upon cocaine-withdrawal. Accordingly, in

one embodiment, a method for identifying an agent to be tested for an ability to prevent or inhibit addiction related-behavior is provided which comprises:

a) exposing a cocaine-addicted subject or brain cells of a cocaine-addicted subject to a candidate agent, wherein the cocaine-addicted subject is undergoing withdrawal;

b) determining a level of expression of at least one gene in the cocaine-addicted subject or brain cells of the cocaine-addicted subject, wherein the at least one gene is identified in Tables 1-15; and

comparing the level of expression of the gene in both the cocaine-addicted subject or brain cells of the cocaine-addicted subject in the presence of the candidate agent with the level of expression of the gene in the cocaine-addicted subject or brain cells of the cocaine-addicted subject in the absence of the candidate agent, wherein a reversal in the level of expression of the gene in the cocaine-addicted subject or brain cells of the cocaine-addicted subject in the presence of the candidate agent relative to the level of expression of the gene in the absence of the candidate agent indicates that the candidate agent is an agent to be tested for the ability to prevent or inhibit addiction related behavior.

[98] In some embodiments of the latter method, if at least one gene is detected the gene does not encode melanocortin 4 receptor.

[99] In another embodiment, a method for identifying an agent to be tested for an ability to prevent or inhibit cocaine addiction-related behavior is provided which comprises:

a) contacting a brain tissue sample from each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

b) detecting a level of expression of at least one gene in both tissue samples, wherein the gene encodes a polypeptide selected from the group consisting of hypertension-regulated vascular factor, myelin-associated basic protein, PB cadherin, calcitonin receptor, melanocortin 4 receptor, ALK-7 kinase and retroposon;

c) subtracting the level of expression of the gene in the sample obtained from the cocaine addiction-free subject from the level of expression of the gene in the sample obtained from the subject having cocaine addiction-related behavior to provide a first value;

d) administering a candidate agent to each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

e) detecting a level of expression of the at least one gene in both tissue samples obtained from the subjects treated with the candidate agent;

f) subtracting the level of expression of the gene in the sample obtained from the treated cocaine addiction-free subject from the level of expression of the gene in the sample obtained from the treated subject having the cocaine addiction-related behavior to provide a second value; and

g) comparing the second value with the first value wherein a decreased second value relative to the first value is indicative of an agent useful in preventing or inhibiting the cocaine addiction-related behavior.

[100] In yet another embodiment, a method for identifying an agent to be tested for an abiity to prevent or inhibit cocaine addiction-related behavior is provided which comprises:

a) obtaining a brain tissue sample from each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

b) detecting a level of expression of at least one gene in both tissue samples, wherein the gene encodes a polypeptide selected from the group consisting of GABA-B receptor subunit gb2, cell adhesion-like molecule, bos taurus-like neuronal axonal protein, similar to mouse chemokine-like factor, FRA-2, similar to human oxygen-regulated protein, similar to mouse mrg1 protein, pentraxin, malic enzyme, olfactomedin-related protein, arc-growth factor enriched in dendrites, protein tyrosine phosphatase, krox, neuritin, microtubule-associated protein 2d and CB1 cannabinoid receptor;

c) subtracting the level of expression of the gene in the sample obtained from the cocaine addiction-free subject from the level of expression of the gene in the sample obtained from the subject having cocaine addiction-related behavior to provide a first value;

d) administering a candidate agent to each of a subject having a cocaine addiction-related behavior and a cocaine addiction-free subject;

e) detecting a level of expression of at least one gene in both tissue samples

obtained from the subjects treated with the candidate agent;

f) subtracting the level of expression of the gene in the sample obtained from the treated cocaine addiction-free subject from the level of expression of the gene in the sample obtained from the treated subject having the cocaine addiction-related behavior to provide a second value; and

g) comparing the second value with the first value wherein an increased second value relative to the first value is indicative of an agent useful in preventing or inhibiting the cocaine addiction-related behavior.

[101] The level of expression of at least one of the disclosed genes in the samples obtained from the subject and disease-free subject and brain cells obtained from the subjects can be detected by measuring either the level of mRNA corresponding to the gene or the protein encoded by the gene. RNA can be isolated from the samples by methods well-known to those skilled in the art as described e.g., in Ausubel et al., Current Protocols in Molecular Biology, Vol. 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John Wiley & Sons, Inc. (1996).

[102] Methods for detecting the level of expression of mRNA are well-known in the art and include, but are not limited to, Northern blotting, reverse transcription PCR, real time quantitative PCR and other hybridization methods.

[103] A particularly useful method for detecting the level of mRNA transcripts obtained from a plurality of the disclosed genes involves hybridization of labeled mRNA to an ordered array of oligonucleotides. Such a method allows the level of transcription of a plurality of these genes, i.e., two or more, to be determined simultaneously to generate gene expression profiles or patterns. The gene expression profile derived from the sample obtained from the subject having the cocaine addiction-related behavior treated with agent can be compared with the gene expression profile derived from the sample obtained from the untreated subject having the cocaine addiction-related behavior to determine whether the genes are up- or down-regulated in the sample from the treated subject relative to the genes in the sample obtained from the untreated subject, and thereby determine whether the agent prevents or inhibits cocaine addition-related behavior.

[104] The oligonucleotides utilized in this hybridization method are bound to a solid support. Examples of solid supports include, but are not limited to, membranes, filters, slides, paper, nylon, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, polymers, polyvinyl chloride dishes, etc. Any solid surface to which the oligonucletides can

be bound, either directly or indirectly, either covalently or non-covalently, can be used. A particularly preferred solid substrate is a high-density array or DNA chip (see "Materials and Methods"; and Example 1). These high density arrays contain a particular oligonucleotide probe in a pre-selected location on the array. Each pre-selected location can contain more than one molecule of the particular probe. Because the oligonucleotides are at specified locations on the substrate, the hybridization patterns and intensities (which together result in a unique expression profile or pattern) can be interpreted in terms of expression levels of particular genes.

[105] The oligonucleotide probes can be labeled with one or more labeling moieties to permit detection of the hybridized probe/target polynucleotide complexes. Label moieties can include compositions that can be detected by spectoscopic, biochemical, photochemical, bioelectronic, immunochemical, electrical optical or chemical means. Examples of labeling moieties include, but are not limited to, radioisotopes, e.g., ^{32}P, ^{33}P, ^{35}S, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectoscopic markers such as fluorescent markers and dyes, linked enzymes, mass spectrometry tags and magnetic labels.

[106] Oligonucleotide probe arrays for expression monitoring can be prepared and used according to techniques which are well-known to those skilled in the art as described, e.g., in Lockhart et al., Nat. Biotech., Vol. 14, pp. 1675-1680 (1996); McGall et al., Proc. Natl. Acad. Sci. USA, Vol. 93, pp. 13555-13460 (1996); and U.S. Patent No. 6,040,138.

[107] Expression of the protein encoded by the gene(s) can be detected by a probe which is detectably labeled, or which can be subsequently labeled. Generally, the probe is an antibody or other ligand which recognizes the expressed protein.

[108] As used herein, the term "antibody" includes, but is not limited to, polyclonal antibodies, monoclonal antibodies, humanized or chimeric antibodies, and biologically functional antibody fragments which are those fragments sufficient for binding of the antibody fragment to the protein.

[109] For the production of antibodies to a protein encoded by one of the disclosed genes, various host animals may be immunized by injection with the polypeptide, or a portion thereof. Such host animals may include, but are not limited to, rabbits, mice and rats, to name but a few. Various adjuvants may be used to increase the immunological

response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances, such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*.

[110] Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals, such as those described above, may be immunized by injection with the encoded protein, or a portion thereof, supplemented with adjuvants as also described above.

[111] Monoclonal antibodies (mAbs), which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler et al., Nature, Vol. 256, pp. 495-497 (1975) and U.S. Patent No. 4,376,110, the human B-cell hybridoma technique (see Kosbor et al., Immunology Today, Vol. 4, p. 72 (1983); Cole et al., Proc. Natl. Acad. Sci. USA, Vol. 80, pp. 2026-2030 (1983); and the EBV-hybridoma technique (see Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

[112] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. USA, Vol. 81, pp. 6851-6855 (1984); Neuberger et al., Nature, Vol. 312, pp. 604-608 (1984); Takeda et al., Nature, Vol. 314, pp. 452-454 (1985)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable or hypervariable region derived from a murine mAb and a human immunoglobulin constant region.

[113] Alternatively, techniques described for the production of single chain antibodies (see U.S. Patent No. 4,946,778; Bird, Science, Vol. 242, pp. 423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA, Vol. 85, pp. 5879-5883 (1988); and Ward et al., Nature, Vol. 334, pp. 544-546 (1989)) can be adapted to produce differentially expressed gene single-chain antibodies. Single-chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single-chain polypeptide.

[114] Most preferably, techniques useful for the production of "humanized antibodies" can be adapted to produce antibodies to the proteins, fragments or derivatives thereof. Such techniques are disclosed in U.S. Patent Nos. 5,932,448; 5,693,762; 5,693,761; 5,585,089; 5,530,101; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,661,016; and 5,770,429.

[115] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed (see Huse et al., Science, Vol. 246, pp. 1275-1281 (1989)) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

[116] The extent to which the known proteins are expressed in the sample is then determined by immunoassay methods which utilize the antibodies described above. Such immunoassay methods include, but are not limited to, dot blotting, Western blotting, competitive and non-competitive protein binding assays, enzyme-linked immunosorbant assays (ELISA), immunohistochemistry, fluorescence-activated cell sorting (FACS) and others commonly used and widely described in scientific and patent literature, and many employed commercially.

[117] Particularly preferred, for ease of detection, is the sandwich ELISA, of which a number of variations exist, all of which are intended to be encompassed by the present invention. For example, in a typical forward assay, unlabeled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex. At this point, a second antibody,

labeled with a reporter molecule capable of inducing a detectable signal, is then added and incubated, allowing time sufficient for the formation of a ternary complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal, or may be quantitated by comparing with a control sample containing known amounts of antigen. Variations on the forward assay include the simultaneous assay, in which both sample and antibody are added simultaneously to the bound antibody, or a reverse assay in which the labeled antibody and sample to be tested are first combined, incubated and added to the unlabeled surface bound antibody. These techniques are well-known to those skilled in the art, and the possibility of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-site technique. For the immunoassays of the present invention, the only limiting factor is that the labeled antibody be an antibody which is specific for the protein expressed by the gene of interest.

[118] The most commonly used reporter molecules in this type of assay are either enzymes, fluorophore- or radionuclide-containing molecules. In the case of an enzyme immunoassay an enzyme is conjugated to the second antibody, usually by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different ligation techniques exist, which are well-known to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, among others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. A solution containing the appropriate substrate is then added to the tertiary complex. The substrate reacts with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an evaluation of the amount of protein which is present in the serum sample. Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody absorbs the light energy, inducing

a state of excitability in the molecule, followed by emission of the light at a characteristic longer wavelength. The emission appears as a characteristic color visually detectable with a light microscope. Immunofluorescence and EIA techniques are both very well-established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent or bioluminescent molecules may also be employed. It will be readily apparent to the skilled artisan how to vary the procedure to suit the required use.

[119] The following examples are included to demonstrate preferred embodiments of the invention.

EXAMPLES

RESEARCH DESIGN AND METHODS

Strategy to Identify Changes in Gene Expression in the NAc Shell and other Brain Regions During Prolonged Abstinence

[120] Six groups of rats (n=10/group) underwent 3 weeks (15 days) of daily (6-10 hours) cocaine self-administration, followed by short or long periods of forced abstinence prior to sacrifice. Changes in gene expression that coincide with time-dependent increases in cocaine-seeking behavior were identified by comparing changes in 1-week abstinence and 1-week extinction groups, as illustrated in Figure 5 below (Groups I and II, respectively). First, a direct comparison between 1-week abstinence and 1-week extinction groups was conducted to identify differences. This allowed detection of genes that correspond to the groups with the greatest differences in drug-seeking behavior. Second, each experimental group (1 week abstinence and extinction) was directly compared to their respective untreated control groups to test whether differences between the groups represent reversals in gene expression between the withdrawal and extinction conditions. Direct comparisons with control groups also allowed detection of genes changed in withdrawal or extinction that might also contribute to drug-seeking behavior though their levels might not necessary be reversed between extinction and withdrawal.

Surgery, Behavioral Testing and Dissection of Specific Brain Regions

[121] Both experimental and control groups consisted of individually housed, male Sprague Dawley rats. Experimental animals were surgically implanted with chronic, indwelling intravenous catheter as follows (see Sutton et al., *supra*). All surgery were

performed under aseptic conditions, in a clean area used solely for surgical procedures. Each surgery was done on a separate, clean sheet of Whatman Benchkote paper. Surgical instruments were autoclaved and cleaned (cleaned and soaked in 70% ethanol between successive surgeries). Rats (at least 300 g) and mice (25-30 g) were anesthetized with an i.p. injection of pentobarbital (1.0 mg/kg; rats) and ketamine/xylazine (10 mL/kg; mice), and penicillin procaine intramuscular (i.m.) (60,000 IU/0.2 mL rats, 6,000 units/0.02 mL mice) was given as a prophylactic. The back area of the animals were shaved and cleaned with 70% ethanol, and 2 incisions were made, one on the back (2 cm), and one on the neck (1 cm). The jugular vein was isolated and a sterile Silastic catheter was inserted to the level sinus just outside the right atrium, and mounted in place with surgical mesh. The remaining catheter was pulled from the neck area subcutaneously back incision. Then the catheter exited via 22-gauge stainless steel tubing cemented into place with dental cement and skull screws on a plastic back mount. The incisions were sutured closed with silk surgical thread and the wounds treated with topical antibiotic, and the animal were given an i.m. injection of penicillin G procaine i.m. (60,000 IU/0.2 mL).

[122] Rats implanted with intravenous (i.v.) catheter recovered from surgery on a warming pad. The rats were not used for experimentation for at least 4 days. During this time, each animal was monitored for distress or infection, and the catheter was flushed daily with 0.2 mL of heparinized saline (20 IU/mL/kg). Because prior exposure to analgesics can alter subsequent behavioral responses to drugs of abuse, rats did not receive post-operative analgesics. Controls remained in their home cages with frequent handling throughout the experiment. Experimental rats were allowed to self-administer cocaine by lever pressing (Fixed-ratio 1: Time-out 10 seconds, 0.5 mg/kg/injection) during their dark cycle 5 days/week for 3 weeks. Each cocaine infusion was delivered over 1.25 seconds concurrent with a cue light, and followed by a 10-second time-out period. The house-light was extinguished during the injection time-out period; together these stimuli constituted the cocaine cue used in reinstatement below. The experimental animals self-administered cocaine in contextually distinct operant chambers located in testing rooms outside the animal colony. During the first week, rats self-administered cocaine for 10 hours/day to hasten acquisition and accustom them to high levels of cocaine exposure. During the second and third weeks, animals self-administered cocaine 6 hours/day. These conditions typically produced self-regulated levels of cocaine intake of 50-60 mg/kg/6-hour test session at the

end of self-administration testing, and more precisely mimic daily patterns of cocaine binges in humans.

[123] Following 3 weeks of cocaine self-administration, animals were divided into experimental groups with equivalent mean levels of cocaine intake, and important factor that determines the propensity for cocaine-seeking during abstinence. Experimental Group II underwent extinction training for 5 days during the first week of abstinence for 6 hours/day, beginning 3 days after their final self-administration test session. Experimental Group IV underwent extinction training for 5 days during their sixth week of abstinence. Responding at both drug-paired and inactive lever were recorded during this time. On the last hour of the final extinction test session, cue-induced reinstatement of cocaine-seeking behavior was tested. During this hour, cues specifically associated with prior cocaine infusions during self-administration (house light off/cue light on) were presented every 2 minutes, and responding at the drug-paired and inactive levers were recorded. Experimental Group I remained in their home cages until sacrifice. Three more experimental groups underwent saline self-administration for 3 weeks and were sacrificed along with Groups I, II and IV. Each group consisted of 6-14 animals to reduce the effects of variability from individuals or dissection procedures on array profiling.

[124] Animals undergoing extinction training were sacrificed 3 days after their last extinction training session; animals remaining in their home cages were sacrificed at similar times during abstinence. Animal were removed from their home cages and immediately sacrificed by decapitation. Brains were rapidly dissected and chilled slices in ice-cold artificial cerebral spinal fluid for 2 minutes. Tissue punches (12- to 16-gauge) were collected from serial coronal brain slices (1.2-1.5 mm thick) based on the locations depicted in Figure 6. A 14-gauge punch was used to collect NAc core samples, and a 12-gauge punch was used to collect a "half moon" slice of the remaining NAc shell tissue, both yielding about 8-10 mg tissue/punch. Punches were rapidly frozen on dry ice, and stored at -80°C until shipped to GNF for the GeneChip studies.

Isolation of Total RNA and Synthesis of cRNA Samples

[125] Total RNA was isolated from pooled tissue samples using Trizol reagent (1 mL Trizol per 50 mg tissue) (Gibco BRL) and a homogenizer (Polytron, Kinematica) run at maximum speed for 90 seconds. The standard Trizol procedure was

used, and RNA after ethanol precipitation was further purified with Rneasy columns (Qiagen). Quality of total RNA was assessed by agarose gel electrophoresis and quantity by spectrophotometer in water and Tris, pH 7.5. Yields were lower than expected and ranged from 4-20 μg. After gel electrophoresis and quantitation, the amount of the limiting sample was 3 μg. Due to the low yield, 250 nanogram aliquots were removed as a preventative measure in case cRNA yields were inadequate and a double amplification of the total RNA was needed. Complementary DNA (cDNA) was synthesized from 3 mg total RNA (corresponding to the amount of the sample with lowest yield) using a T7 promotor/oligo dT primer which allows for subsequent linear amplification of the resulting cDNA (see Van Gelder et al., "Amplified RNA Synthesized From Limited Quantities of Heterogeneous cDNA", Proc. Natl. Acad. Sci. USA, Vol. 87, pp. 1663-1667 (1990)). This procedure results in cDNA and cRNA populations that accurately and reproducibly represent the total RNA of origin (see Lipshutz et al., "High Density Synthetic Oligonucleotide Arrays", Nature Gen., Vol. 21, pp. 20-24 (1999); Lockhart et al., "Expression Monitoring by Hybridization to High-Density Oligonucleotide Arrays", Nature Biotech., Vol. 14, pp. 1675-1680 (1996); and Wodicka et al., "Genome-Wide Expression Monitoring in *Saccharomyces cerevisiae*", Nature Biotech., Vol. 15, pp. 1359-1367 (1997)). Briefly, 3 μg total RNA was used to make first strand cDNA using the Superscript Choice system (Gibco BRL) and a T7 promotor/oligodT primer (Gibco). Second strand cDNA was made with the Superscript Choice system. All of the resulting cDNA, after phenol:chloroform purification and ammonium acetate precipitation, was used as a template to make biotinylated amplified antisense cRNA using T7 RNA polymerase (Enzo kit, Affymetrix). Twenty micrograms cRNA was fragmented to a target range of 20-100 bases in length using fragmentation buffer (200 mM Tris-acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc) and heating for 35 minutes at 94°C. This procedure both reduces secondary structure of cRNA and prevents it from hybridizing to adjacent DNA probes on the array (Lockhart et al., *supra* and Southern et al., "Molecular Interactions on Microarrays", Nature Gen., Vol. 21, pp. 5-9 (1999)). Quality of cRNA and size distribution of fragmented cRNA was examined by both agarose and polyacrylamide gel electrophoresis. It was determined that fragmentation did not yield the expected size range, and further fragmentation resulted in loss of sample. For this reason, the double amplification protocol was used.

Amplification and Labeling of Small Amounts of mRNA

[126] Occasionally, yields of total RNA from small amounts of dissected brain regions is poor in quantity and yet of high quality. Thus, we used double linear amplification procedure as described (see Luo et al., "Gene Expression Profiles of Laser-Captured Adjacent Neuronal Subtypes", [*published erratum appears in Nat. Med., Vol. 5, No. 3, p. 355 (1999)*] Nat. Med., Vol. 5, pp. 117-122 (1999)) and modified for use in our laboratory. First and second stranded cDNA was synthesized as described above using 50 ng starting total RNA, but first, unlabeled cRNA was made using the Megascript kit (Ambion). cRNA was purified with a microcon-50 column (Millipore) and cDNA was again made with random primers and Superscript II (GibcoBRL) at 37°C for 1 hour, incubated at 37°C in the presence of RNAse H (GibcoBRL) for 20 minutes. After heat denaturing the enzymes, a T7-oligo dT primer was added to the mixture and second strand cDNA was made with DNA polymerase I and then T4 DNA polymerase (GibcoBRL). cDNA was purified with microcon-50 columns (Millipore) and a second round of cRNA amplification was performed using the Enzo kit (Affymetrix). Unlike amplification by PCR, this method results in a linear amplification of the total RNA (above references). Between 39 and 84 μg of labeled cRNA was made from 50 ng starting total RNA. Twenty μg cRNA was fragmented as described above, fragmention was successful as determined by gel electrophoresis, and 15 μg fragmented cRNA was added to Affymetrix Gene Chip® Rat Genome U34 arrays with 1 x MES hybridization buffer using standard protocols outlined in the Gene Chip® Expression Analysis Technical Manual (Affymetrix). Hybridization was for 16 hours at 45°C. The same hybridization samples were then removed from the chips and re-hybridized to identical arrays to make duplicates of each sample.

Washing, Staining and Scanning Arrays

[127] Following hybridization of sample to arrays, sample was removed and arrays were washed to remove excess sample. Biotinylated cRNA that is specifically hybridized to the array was stained first with streptavidin phycoerythrin (SAPE, Molecular Probes), then with biotinylated anti-streptavidin antibody, and again with SAPE using standard protocols outlined in the Gene Chip® Expression Analysis Technical Manual (Affymetrix). Following washing, arrays were scanned with a laser scanner (Agilent). After scanning, Gene Chip® software aligns a grid to the image so that individual probe sets can be identified. The quantitative assessment of "present" or "absent" probe sets is based on

the number of instances in which the PM signal is significantly larger than the MM signal across the redundant set of probes for each gene. This array design and analysis scheme is essentially a "voting" scheme. Determination of quantitative RNA abundance is made from the average of the pairwise differences (PM minus MM) across the set of probes for each RNA (average difference value). In order to compare average difference values for each RNA between different arrays, intensity values are scaled (normalized) using intensity values taken over the entire array. The Gene Chip® software makes qualitative calls of "Increase" or "Decrease" and quantitative assessments of the absolute size ("fold change") of any differences. In order to increase confidence in the results, all experiments were performed using duplicate hybridizations. Only differences between duplicates are considered (see below).

Data Filtering to Find Differentially Expressed Genes (Primary Screen)

[128] We have developed a Web-based software tool at our institute for gene expression array data filtering. This tool allows us to filter data with user-defined criteria. For example, if one is comparing gene expression changes between arrays A and B, fold changes are first made between A and B. Fold changes are also measured between duplicate arrays A' and B'. Gene expression changes that are common between the duplicate comparisons are then selected. The criteria for valid differences are as follows:

- Genes scored as "Increased"/"Moderately Increased" or "Decreased"/"Moderately Decreased" (by the standard Affymetrix algorithm) in both comparisons.
- Genes with a minimum 2-fold change in both comparisons, and a minimum absolute change of 50 units in both comparisons.
- Genes scored as "present" in the experimental file or "present" or "moderate" in the baseline file of at least one of the two comparisons.

[129] This software tool can rapidly and accurately manage thousands of potentially regulated genes with a variety of filter settings. The stringency of the filter can be varied depending on the number of potentially regulated genes found. This same data filtering tool can also be used to examine the consistency of the duplicate arrays by finding the number of genes that are significantly "different" between duplicates.

[130] A different data filtering approach was used to find differentially expressed genes in the NAc core, CeA, mPFC and VTA. The reasons for the change in the approach are that the new methods are easily adaptable to our gene expression database and they do not rely on "Increase, Decrease, Absence or Presence" calls generated by the Affymetrix algorithm. The Web-based tool used for finding gene expression changes in the NAc shell is less practical to use.

[131] Two different filters were used to generate data for the NAc core, CeA, mPFC and VTA. The sum of the findings from both filters were used to generate the final gene lists, with redundant entries collapsed to generate one entry per probe set. The first filter used was a one-way ANOVA. Values less than a value of 20 were first forced to a value of 20, then ANOVA was performed.

[132] Probe sets were retained in the gene lists only after they met the following criteria:

1. P-value less than 0.01.
2. Fold change difference between statistical groups at least 1.7.
3. Maximum intensity (average difference value) across the group of at least a value of 200.

[133] The second filter used to generate data for the NAc core, CeA, mPFC and VTA avoided the potential problems of using ANOVA for small sample sizes. First, all values less than a value of 200 were forced to a value of 200. Then, mean values of the groups, standard deviations within the groups, and fold change differences between the groups were calculated and probe sets were retained only if they met the following criteria:

1. Fold change difference between groups at least 1.7.
2. The standard deviation of the group divided by the mean of the same group must have been a value of 0.25 or less for both groups.

Tissue Dissection/Western Blot Procedures

[134] Rats were removed from their homecages and immediately decapitated in a separate room; the brains were rapidly dissected and chilled in ice-cold physiological buffer (5 mM KCl, 126 mM NaCl, 1.25 mM NaH_2PO_4, 10 mM D-glucose, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgSO_4$, pH 7.4). NAc core samples were obtained with a 14-gauge punch from chilled coronal brain slices (0.7-2.2 mM anterior to bregma; Paxinos et al.

(1998)), and immediately frozen and stored at -80°C. Half moon-shaped NAc shell samples were obtained with a 12-gauge punch of the remaining ventral-medial shell tissue.

[135] Tissue samples were homogenized by sonication in 350 μL (NAc) of 1% SDS. Protein concentrations were determined (Lowry et al. (1951)), and 10 μg protein/sample was subjected to SDS-polyacrylamide gel electrophoresis (7.5-10% acrylamide/0.12% bisacrylamide), followed by electrophoretic transfer to nitrocellulose (Bio-Rad, Hercules, CA). Proteins were immunolabeled overnight at 4 x in blocking buffer consisting of 5% non-fat dried milk powder in PBST (10 mM sodium phosphate, pH 7.4, 0.9% NaCl, 0.1% Tween-20). Following incubation with the primary antibody, blots were washed with blocking buffer, and incubated for 2 hours at 20°C with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:2000; Chemicon, Temecula, CA) in PBST. The blots were washed again in PBST, and immunoreactivity visualized using enhanced chemiluminescense for peroxidase labeling (New England Nuclear, Boston, MA). Protein immunoreactivity was quantified by densitometric analysis using NIH Image 1.57 (National Institute of Health, Bethesda, MD). TH immunoreactivity was linear over a 4-fold range of tissue concentrations under these conditions.

Data Analysis

[136] Each gel contained 7-11 control samples alternating with samples from experimental animals. To normalize data from different gels, protein immunoreactivity for each control and experimental sample was expressed as a percentage of the mean control value for that particular gel. For statistical analysis, age- and batch-matched control values were pooled into a single group, and compared with 2 cocaine-trained groups with 1-way ANOVA. Post-hoc comparisons were made among control and cocaine-trained groups with Newman Keuls tests.

[137] Analysis of data from nucleus accumbens core, central nucleus of the amygdala, medial prefrontal cortex, and ventral tegmental area indicated that the 1 week withdrawal control and 1 week extinction control groups were not equivalent. Therefore pooling all of the control values into a single control group was not valid for these comparisons. Instead, extinction and withdrawal groups were compared directly or to their respective controls.

Example 1

Identification of Extinction/Withdrawal Differences in Gene Expression in the Nac Shell and Other Brain Regions During Prolonged Abstinence Using Gene Expression Profiling

[138] The advent of oligonucleotide arrays increases the feasibility of forward genetic approaches to identify gene regulation in studies of complex behaviors. This technology replaces more cumbersome methods of subtraction hybridization and differential display with the advantage of profiling thousands of genes simultaneously. Figure 4 illustrates 2 candidate genes identified in our preliminary studies from contralateral NAc shell tissue samples taken from animals used in the extinction studies described above. These genes were selected by comparing 1-week extinction training and 1-week withdrawal groups according to stringent criteria described in the Research Design and Methods section. The top panel illustrates a 3.7-fold difference in expression of a retroviral derived gene retroposon (see Table 1). This gene is over-expressed in withdrawal from cocaine self-administration (88%), but down-regulated (49%) in animals that experienced extinction training when compared to untreated age- and batch-matched controls. In contrast, expression of the CB1 cannabinoid receptor gene is reduced (53%) in withdrawal, but normalized to near control levels following extinction training. Tables 1-15 contain all of the genes selected by both primary and secondary screening procedures for this comparison (see "Methods"). This procedure employs control/control comparisons to eliminate false positives, in addition to the gene filtering software-based selection procedure. As shown in Table 1, there are several genes for structural proteins (i.e., PB cadherin, microtubule-associated protein) suggesting neuroplasticity in neuronal contacts (dendritic spines and arborization). There are also 4 gene candidates (highlighted in bold) that already are implicated in drug reward and addiction. For example, GABA B receptor agonists have been proposed as a possible pharmacotherapy for cocaine addiction, and CB1 cannabinoid receptors mediate central effects of cannabis, and can modulate dopaminergic responses in striatum. Similarly, FRA2, is a Fos-Related Antigen like ΔFosB, which has been implicated in sensitivity to cocaine (see Kelz et al., "Expression of the Transcription Factor ΔFosB in the Brain Controls Sensitivity to Cocaine", Nature, Vol. 401, pp. 272-276 (1999). The melanocortin receptor MC4 has recently been shown to be up-regulated during withdrawal from repeated cocaine treatments, and intra-NAc infusions of an MC4 antagonist reverse the

rewarding effects of cocaine to produce a cocaine aversion instead in a place preference paradigm (see Taylor et al., "Role of Melanocortin in Drug Reward", submitted).

Table 1. Effects of Extinction Training on Gene Expression in the NAc Shell Following 1 Week Withdrawal from Cocaine Self-Administration

Gene Name	1 Week Withdrawal*	1 Week Extinction*	Extinction vs. Withdrawal	Genbank Accession No.
GABA-B receptor subunit gb2	↓ 30%	↑ 48%	2.12-fold Δ	AJ011318.1
Hypertension-regulated vascular factor	↑ from 0	0	Normalized	AF055714
Myelin-associated basic protein	↑ 140%	↓ 7%	2.59-fold Δ	X87900.1
PB cadherin	↑ 17%	↓ 56%	2.17-fold Δ	D83349.1
Calcitonin receptor	↑ 33%	↓ 80%	6.58-fold Δ	L13041.1
Cell adhesion-like molecule	↓ 88%	↑ 6%	8.92-fold Δ	M88709.1
Bos taurus-like neuronal axonal protein	↓ 36%	↑ 34%	2.08-fold Δ	U92535.1
Similar to mouse chemokine-like factor	↓ 47%	↑ 65%	2.08-fold Δ	AF144754.1
FRA-2	↓ 66%	↑ 41%	3.21-fold Δ	X98051.1
Similar to human oxygen regulated protein	↓ 37%	↑ 46%	2.32-fold Δ	AI009098
Similar to mouse mrg1 protein	↓ 48%	↑ 87%	3.62-fold Δ	AI014091
Pentraxin	↓ 70%	↑ 63%	5.41-fold Δ	U18772
Malic enzyme	↓ 33%	↑ 61%	2.39-fold Δ	M26594.1
Olfactomedin related protein	↓ 38%	↑ 48%	2.40-fold Δ	U03414
Arc – growth factor enriched in dendrites	↓ 45%	↑ 21%	2.18-fold Δ	U19866.1
Protein tyrosine phosphatase	↓ 55%	↑ 13%	2.49-fold Δ	U28938
Melanocortin 4 receptor	↑ 272%	↓ 35%	4.21-fold Δ	U67863.1
ALK-7 kinase	↑ 44%	↓ 44%	2.57-fold Δ	U69702.1
Krox	↓ 47%	↑ 15%	2.19-fold Δ	U75397
Neuritin	↓ 87%	↑ 28%	10.1-fold Δ	U88958.1
Microtubule-associated protein 2d	↓ 17%	↑ 67%	2.02-fold Δ	X74211.1
CB1 cannabinoid receptor	↓ 53%	↑ 19%	2.52-fold Δ	X55812.1
Retroposon	↑ 88%	↓ 49%	3.70-fold Δ	U83119.1

*Expressed as % Δ from mean control value for both groups (n = 5-8 pooled samples/group). Genes selected according to procedure described in Research Design and Methods. Gene names in bold indicate gene products in the NAc implicated in drug reward or addiction. Only changes in known genes are shown. Genes are selected based on criteria (see Methods) where both duplicate comparisons between extinction and withdrawal groups exceed 2-fold and are directionally similar. Base on this primary selection procedure, a secondary selection procedure eliminates genes when average duplicate values from both control groups vary more than 20% from the overall mean of the control groups. For genes expressed in low levels (<100 densitometric units), all control values must lie within 25 units of the overall mean. Average difference values for all groups and their respective control groups are shown in the Appendix tables.

[139] Thus, this latter neuroadaptation represents one difference replicated by alternative means (*in situ*). Several other genes regulated by withdrawal but not modified by extinction, and by extinction training alone are shown in Tables 2-16 below. These results demonstrate oligonucleotide detection of extinction/withdrawal differences.

Table 2. Average Difference Values for 1-Week Extinction Versus 1-Week Extinction Controls

Probe Set	Gene Name	1-Week Extinction Control	1-Week Extinction
AF050659UTR#1_at	Activity and neurotransmitter-induced early 7 mRNA	269	114
AF050659UTR#1_at		347	132
AJ000485_at	CLIP-115 protein	95	168
AJ000485_at		40	153
AJ006971_g_at	DAP-like kinase	184	545
AJ006971_g_at		209	641
D83348_at	Long-type PB cadherin	113	285
D83348_at		135	298
K02248cds_s_at	Somatostatin-14 gene	69	365
K02248cds_s_at		132	460
M13100cds#3_f_at	Long interspersed repetitive DNA sequence	730	348
M13100cds#3_f_at		938	474
M16410_at	Neurokinin B precursor	117	262
M16410_at		110	241
M32062_at	Fcgamma receptor	-19	96
M32062_at		20	75
M55015cds_s_at	Nucleolin gene	49	154
M55015cds_s_at		66	147
M89646_g_at	Ribosomal protein S24	665	1466
M89646_g_at		765	1370
rc_AA799406_at	Genes for 18S, 5.8S and 28S ribosomal rRNAs	244	683
rc_AA799406_at		-42	577
rc_AA800039_s_at	Unknown	346	667
rc_AA800039_s_at		264	667
rc_AA866419_at	Unknown	59	150
rc_AA866419_at		-26	109
rc_AA875268_at	Similar to B.taurus PSST subunit of	683	1332
rc_AA875268_at	NADH:ubiquinone oxidoreduc	655	1361
rc_AA891727_g_at	Unknown	250	542
rc_AA891727_g_at		285	576
rc_AA891796_at	1-cys peroxiredoxin; thiol-specific antioxidant protein	412	889
rc_AA891796_at		557	1180
rc_AA892041_at	Homosapiens over-expressed breast tumor	768	1481
rc_AA892041_at	protein mRNA	788	1482
rc_AA892123_at	Ribosomal protein L36	280	708
rc_AA892123_at		378	761
rc_AA892864_at	Unknown	54	264
rc_AA892864_at		-2	259
rc_AA924772_at	Growth inhibitory factor-metallothionein homolog	1533	2979
rc_AA924772_at		1577	3108

Table 2 (cont'd)

Probe Set	Gene Name	1-Week Extinction Control	1-Week Extinction
rc_AI010581_at	11 Kd diazepam binding inhibitor	249	569
rc_AI010581_at		246	570
rc_AI014135_g_at	CDK103	822	340
rc_AI014135_g_at		916	317
rc_AI171844_at	F1-aTPase epsilon subunit	563	1232
rc_AI171844_at		564	1345
rc_AI176460_s_at	32S pre-rRNA 5' terminal part with 28S rRNA sequence	1640	3545
rc_AI176460_s_at		1728	3573
rc_AI227887_at	Similar to Mus musculus CDC42 mRNA	304	7
rc_AI227887_at		334	119
rc_AI639367_at	Unknown	574	63
rc_AI639367_at		605	81
rc_AI639521_at	Unknown	141	2
rc_AI639521_at		109	21
U75392_s_at	B-cell receptor associated protein 37	191	516
U75392_s_at		190	488
X02002_at	Thy-1 gene for cell surface glycoprotein	197	482
X02002_at		251	498
X05472cds#1_s_at	2.4 Kb repeat DNA right terminal region	428	169
X05472cds#1_s_at		311	123
X14671cds_s_at	Liver mRNA for ribosomal protein L26	871	1811
X14671cds_s_at		1050	1949
X53581cds#5_f_at	Long interspersed repetitive DNA sequence	460	125
X53581cds#5_f_at		425	109
X55153mRNA_s_at	RP2 gene for ribosomal protein P2	723	1603
X55153mRNA_s_at		596	1639
X56325mRNA_s_at	Alpha-1 globin gene	1886	3834
X56325mRNA_s_at		1848	4115
X61295cds_s_at	L1 retroposon mRNA	1299	635
X61295cds_s_at		1080	529
X62952_at	Vimentin	-60	117
X62952_at		26	117
X63594cds_g_at	RL/IF-1	-32	121
X63594cds_g_at		48	194
X68283_at	Ribosomal protein L29	703	1462
X68283_at		524	1271
Y13714_at	Osteonectin	174	531
Y13714_at		187	505

[140] Genes that passed the filtering criteria outlined above for the nucleus accumbens shell are listed. Average difference values (from GeneChip version 3.2) are listed for each gene from each duplicate chip from both the 1 week extinction and 1 week extinction control groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 3. Average Difference Values for 1-Week Extinction, 1-Week Withdrawal and Their Corresponding Control Groups

Probe set no.	Gene name	1-week extinction	1-week withdrawal	1-week extinction control	1-week withdrawal control
AF055714UTR#1_at	Hypertension-regulated	-14	63	-22	-22
AF055714UTR#1_at	vascular factor	-14	55	-23	-3
AF058795_at	GABA-B receptor subunit gb2	621	309	432	468
AF058795_at		695	311	456	421
D28111_at	Myelin-associated basic	995	2551	879	1037
D28111_at	protein	835	2186	783	1243
D83349_at	PB cadherin	1709	3903	2960	3704
D83349_at		1798	3703	2580	3759
L13040_s_at	Calcitonin receptor	11	149	124	83
L13040_s_at		32	134	129	91
M13100cds#1_at	Long repetitive sequence	895	2774	237	1335
M13100cds#1_at		910	3278	414	1220
M13100cds#1_g_at	Long repetitive sequence	117	297	1564	270
M13100cds#1_g_at		84	293	1181	375
M13100cds#2_s_at	Long repetitive sequence	177	892	195	408
M13100cds#2_s_at		181	781	174	355
M13100cds#3_f_at	Long repetitive sequence	348	926	547	1001
M13100cds#3_f_at		474	1206	483	974
M13100cds#4_f_at	Long repetitive sequence	153	547	730	229
M13100cds#4_f_at		89	420	938	192
M13100cds#5_s_at	Long repetitive sequence	212	802	249	390
M13100cds#5_s_at		157	765	175	342
M13100cds#6_f_at	Long repetitive sequence	273	916	425	766
M13100cds#6_f_at		185	626	384	771
M13101cds_f_at	Unknown	57	371	746	307
M13101cds_f_at		153	588	612	439
M88709_at	Cell adhesion-like molecule	341	65	262	264
M88709_at		230	-1	212	339
rc_AA799423_at	Unknown	79	292	172	168
rc_AA799423_at		71	201	193	244
rc_AA799448_g_at	Unknown	470	90	392	385
rc_AA799448_g_at		462	218	489	347
rc_AA799594_at	Unknown	1974	3970	1692	1572
rc_AA799594_at		1497	3251	2238	2138
rc_AA859536_at	Similar to Bos taurus neuronal	3524	1672	2666	2273
rc_AA859536_at	axonal membrane protein	3517	1707	2885	2675
rc_AA874803_g_at	Similar to mouse chemokine-	1515	488	896	802
rc_AA874803_g_at	like factor	1485	479	1023	907
rc_AA875001_at	Unknown	255	-72	213	221
rc_AA875001_at		270	24	224	278
rc_AA875032_at	FRA-2	285	102	193	222
rc_AA875032_at		344	94	263	214
rc_AI009098_at	Highly similar to human	612	292	491	390
rc_AI009098_at	oxygen-regulated protein	537	204	344	346
rc_AI014091_at	Highly similar to mouse mrg1	231	84	36	195
rc_AI014091_at	protein (a cytokine-inducible transcr.	269	54	138	165

Table 3 (cont'd)

Probe set no.	Gene name	1-week extinction	1-week withdrawal	1-week extinction control	1-week withdrawal control
rc_AI014135_g_at	CDK103	340	-20	822	332
rc_AI014135_g_at		317	27	916	368
rc_AI072943_at	Pentraxin	167	48	68	51
rc_AI072943_at		55	-7	51	103
rc_AI073204_at	14-33 protein epsilon	1793	561	1398	440
rc_AI073204_at		1535	587	1340	448
rc_AI171506_at	Malic enzyme	95	28	82	79
rc_AI171506_at		118	61	54	50
rc_AI176710_at	Nuclear orphan receptor	358	62	144	272
rc_AI176710_at		305	54	163	252
rc_AI231445_at	Lysosomal glycoprotein	-80	17	2	17
rc_AI231445_at		-12	39	31	4
rc_AI233362_at	Unknown	919	2280	1405	1088
rc_AI233362_at		1045	2321	1359	1073
rc_AI639088_s_at	Unknown	116	377	353	290
rc_AI639088_s_at		92	350	267	251
rc_AI639118_at	Unknown	143	70	98	119
rc_AI639118_at		130	43	94	128
rc_AI639226_at	Unknown	28	91	65	81
rc_AI639226_at		17	73	90	80
rc_AI639367_at	Unknown	63	530	574	553
rc_AI639367_at		81	453	605	405
rc_AI639484_at	Unknown	1520	509	1243	1265
rc_AI639484_at		1539	612	1194	1385
rc_AI639521_at	Alpha beta crystalline gene	2	99	141	103
rc_AI639521_at		21	84	109	141
rc_H31118_at	Unknown	1247	430	1152	810
rc_H31118_at		1227	492	1176	842
U03414_s_at	Olfactomedin-related protein	1183	534	797	785
U03414_s_at		1194	458	907	731
U03416_at	Olfactomedin-related protein	1184	471	803	737
U03416_at		1186	508	846	859
U19866_at	Arc - a growth factor enriched	815	403	683	594
U19866_at	in dendrites	627	257	549	557
U28938_at	Protein tyrosine phosphatase	461	184	320	416
U28938_at		440	178	426	435
U67863_at	Melanocortin 4 receptor	14	125	21	38
U67863_at		39	98	56	49
U69702_at	ALK-7 kinase	67	188	140	128
U69702_at		80	190	125	132
U75397UTR#1_s_at	Krox	1077	461	964	983
U75397UTR#1_s_at		1010	494	887	793
U83119_f_at	Repetitive DNA sequence	68	393	314	730
U83119_f_at		38	426	379	484
U88958_at	Neuritin	260	40	244	216
U88958_at		257	11	158	192
U95920_at	Precentriolar material	107	233	157	161
U95920_at		102	200	-32	129
X01118_at	Atrial natriuretic polypeptie	109	-15	-34	40
X01118_at		124	-17	10	12
X05472cds#1_s_at	Repeat DNA	169	624	428	422
X05472cds#1_s_at		123	633	311	317

Table 3 (cont'd)

Probe set no.	Gene name	1-week extinction	1-week withdrawal	1-week extinction control	1-week withdrawal control
X05472cds#2_at	Repeat DNA	660	1396	931	630
X05472cds#2_at		630	1412	807	627
X05472cds#3_f_at	Repeat DNA	133	968	213	188
X05472cds#3_f_at		100	878	210	195
X07686cds_s_at	Repeat DNA	58	291	121	135
X07686cds_s_at		28	275	112	112
X17682_s_at	Microtubule-associated	649	319	352	414
X17682_s_at	protein	596	298	335	388
X53455cds_s_at	Microtubule-associated	225	33	126	217
X53455cds_s_at	protein	299	76	53	161
X53581cds#5_f_at	Repeat DNA	125	366	460	411
X53581cds#5_f_at		109	471	425	768
X55812complete_seq_at	CB1 Cannabinoid receptor	294	99	208	251
X55812complete_seq_at		268	124	247	240
X61295cds_s_at	Retroposon	635	2177	1299	1181
X61295cds_s_at		529	2128	1080	1022

[141] Genes that passed the filtering criteria outlined above for the nucleus accumbens shell are listed. Average difference values (from GeneChip version 3.2) are listed for each gene from each duplicate chip from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 4. CeA 1-Week Extinction to Control

Probe set no.	Description	1-Week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
AB016161cds_i_at	AB016161cds Rattus norvegicus mRNA for GABAB receptor 1d, complete cds	352	460	50	234	406	142	0.349754	-2.9
AF010466_s_at	AF010466 Rattus norvegicus interferon gamma (IFN-gamma) mRNA, complete cds	13	-24	298	411	-5.5	354.5	-64.4545	at least 2-fold
AF031430_at	AF031430 Rattus norvegicus syntaxin 7 mRNA, complete cds	227	229	103	119	228	111	0.486842	-2.1
AF042830_at	AF042830 Rattus norvegicus proto-oncogene tyrosine kinase receptor Ret (c-ret) mRNA, partial cds	433	361	253	206	397	229.5	0.578086	-1.7
AF102552_s_at	AF102552 Rattus norvegicus 270 kDa ankyrin G isoform mRNA, partial cds	416	499	216	238	457.5	227	0.496175	-2.0
D13962_g_at	D13962 RATGLUT3 Rat mRNA for neuron glucose transporter	358	341	177	149	349.5	163	0.466381	-2.1
D17711cds_s_at	D17711cds RATCSBP Rat mRNA for dC-stretch binding protein (CSBP), complete cds	301	296	159	149	298.5	154	0.515913	-1.9
D21800_g_at	D21800 RATPSRC10 Rat mRNA for proteasome subunit RC10-II, complete cds	110	114	269	252	112	260.5	2.325893	2.3
D26154UTR#1_at	D26154UTR#1 RATRB109 Rat mRNA for RB109 (brain specific protein), complete cds	532	427	286	220	479.5	253	0.527633	-1.9
D26500_at	D26500 RATDLP9A Rat mRNA for dynein-like protein 9A, partial cds	277	271	137	161	274	149	0.543796	-1.8
D82071_at	D82071 Rattus norvegicus mRNA for hematopoietic prostaglandin D synthase, complete cds / cds = 192,791 / gb = D82071 / gi = 2558504 / ug = Rn.10837 / len = 1004	207	196	94	81	201.5	87.5	0.434243	-2.3
E13644cds_s_at	E13644cds cDNA encoding Neurodap-1 which is located at the post-synaptic membrane thickening regions of neurons and contains RING-H2 finger motif	313	292	151	165	302.5	158	0.522314	-1.9
J00771_at	J00771 RATPRNASE Rat pancreatic ribonuclease mRNA	173	139	430	353	156	391.5	2.509615	2.5
L07398_at	L07398 RATIGVCL Rattus norvegicus (hybridoma 56R-3) immunoglobulin rearranged gamma-chain mRNA variable (V) region, partial cds	670	687	290	280	678.5	285	0.420044	-2.4

Table 4 (cont'd)

Probe set no.	Description	1-Week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
M12112mRNA#3_s_at	M12112mRNA#3 RATANGA2 Rat angiotensinogen mRNA, 3' flank	347	244	502	657	295.5	579.5	1.961083	2.0
M34331_at	M34331 Rat 60S ribosomal subunit protein L35 mRNA, complete cds / cds = 47,418 / gb = M34331 / gi = 206729 / ug = Rn.3458 / len = 451	733	704	1155	1508	718.5	1331.5	1.853166	1.9
rc_AI639304_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx00157 3', mRNA sequence [Rattus norvegicus]	542	524	301	325	533	313	0.587242	-1.7
rc_AA799489_g_at	rc_AA799489 EST188986 Rattus norvegicus cDNA, 3' end / clone = RHEAB66 / clone_end = 3' / gb = AA799489 / gi = 2862444 / ug = Rn.6193 / len= 646	108	-84	373	483	12	428	35.66667	35.7
rc_AA799498_at	rc_AA799498 EST188995 Rattus norvegicus cDNA, 3' end / clone = RHEAB76 / clone_end = 3' / gb = AA799498 / gi = 2862453 / ug = Rn.3835 / len = 683	375	495	44	47	435	45.5	0.104598	-9.6
rc_AA800549_at	rc_AA800549 EST190046 Rattus norvegicus cDNA, 3' end / clone = RLUAB29 / clone_end = 3' / gb = AA800549 / gi = 2863504 / ug = Rn.22957 / len = 491	275	316	461	655	295.5	558	1.888325	1.9
rc_AA800882_g_at	rc_AA800882 EST190379 Rattus norvegicus cDNA, 3' end / clone = RLUAM60 / clone_end = 3' / gb = AA800882 / gi = 2863837 / ug = Rn.24136 / len = 379	204	166	436	403	185	419.5	2.267568	2.3
rc_AA818114_at	rc_AA818114 UI-R-A0-am-g-03-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-A0-am-g-03-0-UI / clone_end = 3' / gb = AA818114 / gi = 2887994 / ug = Rn.7181 / len = 556	210	227	107	101	218.5	104	0.475973	-2.1
rc_AA851403_at	rc_AA851403 EST194171 Rattus norvegicus cDNA, 3' end / clone = RPLAG17 / clone_end = 3' / gb = AA851403 / gi = 2938943 / ug = Rn.3383 / len = 393	474	453	296	209	463.5	252.5	0.544768	-1.8
rc_AA859643_at	rc_AA859643 UI-R-E0-bs-a-08-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bs-a-08-0-UI / clone_end = 3' / gb = AA859643 / gi = 2949163 / ug = Rn.32 / len = 482	597	468	243	137	532.5	190	0.356808	-2.8
rc_AA875659_s_at	rc_AA875659 UI-R-E0-ct-h-07-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-ct-h-07-0-UI / clone_end = 3' / gb = AA875659 / gi = 2980607 / ug = Rn.10966 / len = 424	157	285	390	485	221	437.5	1.979638	2.0

Table 4 (cont'd)

Probe set no.	Description	1-Week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
rc_AA891222_at	rc_AA891222 EST195025 Rattus norvegicus cDNA, 3' end / clone = RHEAQ71 / clone_end = 3' / gb = AA891222 / gi = 3018101 / ug = Rn.1014 / len = 568	380	322	150	98	351	124	0.353276	-2.8
rc_AA891940_at	rc_AA891940 EST195743 Rattus norvegicus cDNA, 3' end / clone = RKIAI82 / clone_end = 3' / gb = AA891940 / gi = 3018819 / ug = Rn.3508 / len = 523	52	212	385	426	132	405.5	3.07197	3.1
rc_AA894292_at	rc_AA894292 EST198095 Rattus norvegicus cDNA, 3' end / clone = RSPAW06 / clone_end = 3' / gb = AA894292 / gi = 3021171 / ug = Rn.19450 / len = 599	441	319	215	222	380	218.5	0.575	-1.7
rc_AA924772_at	rc_AA924772 UI-R-A1-eb-f-02-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-A1-eb-f-02-0-UI / clone_end = 3' / gb = AA924772 / gi = 3071908 / ug = Rn.11325 / len = 372	98	266	499	691	182	595	3.269231	3.3
rc_AI070108_at	rc_AI070108 UI-R-Y0-lu-a-09-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-Y0-lu-a-09-0-UI / clone_end = 3' / gb = AI070108 / ug = Rn.16863 / len = 529	377	336	164	121	356.5	142.5	0.399719	-2.5
rc_AI137421_at	rc_AI137421 UI-R-C2p-ok-c-12-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone=UI-R-C2p-ok-c-12-0-UI / clone_end = 3' / gb = AI137421 / ug = Rn.1485 / len = 556	163	193	442	479	178	460.5	2.587079	2.6
U04934_s_at	U04934 RNU04934 Rattus norvegicus Sprague-Dawley (CD-1) clone Kc1 Na-Ca exchanger mRNA, partial cds	435	421	147	284	428	215.5	0.503505	-2.0
U75899mRNA_g_at	U75899mRNA RNU75899 Rattus norvegicus HSPB2 gene, complete cds	791	664	378	462	727.5	420	0.57732	-1.7
X58830_at	X58830 Rat vgr mRNA / cds = 0,623 / gb = X58830 / gi = 57475 / ug = Rn.10436 / len = 1241	503	484	275	277	493.5	276	0.559271	-1.8
Z50052_at	Z50052 R.norvegicus mRNA for C4BP beta chain protein / cds = 265,1041 / gb = Z50052 / gi = 899381 / ug = Rn.11151 / len = 1091	214	232	71	69	223	70	0.313901	-3.2

Genes that passed the filtering criteria outlined above for differential expression between 1 week extinction and its corresponding control group in the CeA. Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 5. CeA 1-Week Extinction to Withdrawal

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
AB016161cds_i_at	AB016161cds Rattus norvegicus mRNA for GABAB receptor 1d, complete cds	352	460	50	234	406	142	0.349754	-2.9
AB000517_s_at	AB000517 Rattus sp. mRNA for CDP-diacylglycerol synthase, complete cds	300	387	162	146	343.5	154	0.448326	-2.2
AF015304_at	AF015304 Rattus norvegicus equilbrative nitrobenzylthioinosine-sensitive nucleoside transporter mRNA, complete cds / cds = 4,1377 / gb = AF015304 / gi = 2656136 / ug = Rn.5814 / len = 1766	434	419	201	200	426.5	200.5	0.470106	-2.1
AF041373_s_at	AF041373 Rattus norvegicus clathrin assembly protein short form (CALM) mRNA, complete cds / cds = 25,1818 / gb = AF041373 / gi = 2792499 / ug = Rn.10888 / len = 1921	468	405	190	8	436.5	99	0.226804	-4.4
AF064856_at	AF064856 Rattus sp. 7acomp protein mRNA, complete cds	332	244	514	540	288	527	1.829861	1.8
E00775cds_s_at	E00775cds cDNA encoding rat cardionatrin precursor	223	261	-84	-133	242	-108.5	-0.44835	2.2
J00771_at	J00771 RATPRNASE Rat pancreatic ribonuclease mRNA	-50	-13	430	353	-31.5	391.5	-12.4286	at least 2 fold
J05167_at	J05167 Rat band 3 Cl-/HCO_3 exchanger (B3RP3) mRNA, complete cds / cds = 34,3717 / gb = J05167 / gi = 203088 / ug = Rn.9859 / len = 3877	512	412	164	159	462	161.5	0.349567	-2.9
K00996mRNA_s_at	K00996mRNA RATCYP45E Rat cytochrome p-450e (phenobarbital-induced) mRNA, 3' end	200	236	386	368	218	377	1.729358	1.7
L07380_g_at	L07380 RATGHRFRG Rattus rattus (clone pGR2) growth hormone-releasing factor receptor mRNA sequence	375	435	236	227	405	231.5	0.571605	-1.7
L07398_at	L07398 RATIGVCL Rattus norvegicus (hybridoma 56R-3) immunoglobulin rearranged gamma-chain mRNA variable (V) region, partial cds	723	611	290	280	667	285	0.427286	-2.3
M10140_at	M10140 Rat skeletal muscle creatine kinase composite mRNA, complete cds / cds = 69,1214 / gb = M10140 / gi = 203477 / ug = Rn.10756 / len = 1410	43	81	345	410	62	377.5	6.08871	6.1

Table 5 (cont'd)

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
M32754cds_s_at	M32754cds RATINHBAB1 Rat inhibin alpha-subunit gene, exon 1	297	256	578	655	276.5	616.5	2.229656	2.2
M80826_at	M80826 Rat intestinal trefoil protein mRNA, complete cds / cds = 17,262 / gb = M80826 / gi = 207446 / ug = Rn.9960 / len = 431	790	787	70	-10	788.5	30	0.038047	-26.3
rc_AI639304_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx00157 3', mRNA sequence [Rattus norvegicus]	573	503	301	325	538	313	0.581784	-1.7
rc_AA799581_at	rc_AA799581 EST189078 Rattus norvegicus cDNA, 3' end / clone = RHEAC77 / clone_end = 3' / gb = AA799581 / gi = 2862536 / ug = Rn.6207 / len = 569	429	462	209	179	445.5	194	0.435466	-2.3
rc_AA800211_at	rc_AA800211 EST189708 Rattus norvegicus cDNA, 3' end / clone = RHEAM49 / clone_end = 3' / gb = AA800211 / gi = 2863166 / ug = Rn.6299 / len = 740	164	224	326	400	194	363	1.871134	1.9
rc_AA800549_at	rc_AA800549 EST190046 Rattus norvegicus cDNA, 3' end / clone = RLUAB29 / clone_end = 3' / gb = AA800549 / gi = 2863504 / ug = Rn.22957 / len = 491	306	333	461	655	319.5	558	1.746479	1.7
rc_AA800749_at	rc_AA800749 EST190246 Rattus norvegicus cDNA, 3' end / clone = RLUAL02 / clone_end = 3' / gb = AA800749 / gi = 2863704 / ug = Rn.1897 / len = 637	532	392	234	193	462	213.5	0.462121	-2.2
rc_AA859680_g_at	rc_AA859680 UI-R-E0-bs-d-12-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bs-d-12-0-UI / clone_end = 3' / gb = AA859680 / gi = 2949200 / ug = Rn.22632 / len = 437	2002	1841	944	761	1921.5	852.5	0.443664	-2.3
rc_AA874874_at	rc_AA874874 UI-R-E0-ci-d-12-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-ci-d-12-0-UI / clone_end = 3' / gb = AA874874 / gi = 2979822 / ug = Rn.3157 / len = 513	761	632	1099	1322	696.5	1210.5	1.737976	1.7
rc_AA874919_at	rc_AA874919 UI-R-E0-ck-g-09-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-ck-g-09-0-UI / clone_end = 3' / gb = AA874919 / gi = 2979867 / ug = Rn.3174 / len = 542	541	490	216	226	515.5	221	0.42871	-2.3
rc_AA875127_g_at	rc_AA875127 UI-R-E0-bu-d-05-0-UI.s2 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bu-d-05-0-UI / clone_end = 3' / gb = AA875127 / gi = 2980075 / ug = Rn.18698 / len = 579	395	382	208	199	388.5	203.5	0.52381	-1.9

Table 5 (cont'd)

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
rc_AA891690_at	rc_AA891690 EST195493 Rattus norvegicus cDNA, 3' end / clone = RKIAF58 / clone_end = 3' / gb = AA891690 / gi = 3018569 / ug = Rn.22701 / len = 446	167	189	391	335	178	363	2.039326	2.0
rc_AA891940_at	rc_AA891940 EST195743 Rattus norvegicus cDNA, 3' end / clone = RKIAI82 / clone_end = 3' / gb = AA891940 / gi = 3018819 / ug = Rn.3508 / len = 523	109	29	385	426	69	405.5	5.876812	5.9
rc_AA892378_g_at	rc_AA892378 EST196181 Rattus norvegicus cDNA, 3' end / clone = RKIAP70 / clone_end = 3' / gb = AA892378 / gi = 3019257 / ug = Rn.1298 / len = 589	959	890	1732	1866	924.5	1799	1.945917	1.9
rc_AA944423_at	rc_AA944423 EST199922 Rattus norvegicus cDNA, 3' end / clone = REMAJ02 / clone_end = 3' / gb = AA944423 / gi = 3104339 / ug = Rn.6165 / len = 670	435	376	255	200	405.5	227.5	0.561036	-1.8
rc_AA946384_at	rc_AA946384 EST201883 Rattus norvegicus cDNA, 3' end / clone = RLUBH49 / clone_end = 3' / gb = AA946384 / gi = 3106300 / ug = Rn.11301 / len = 576	464	624	352	278	544	315	0.579044	-1.7
rc_AI102868_g_at	rc_AI102868 EST212157 Rattus norvegicus cDNA, 3' end / clone = REMBT90 / clone_end = 3' / gb = AI102868 / ug = Rn.221 / len = 489	1431	1441	702	953	1436	827.5	0.576253	-1.7
rc_AI228599_at	rc_AI228599 EST225294 Rattus norvegicus cDNA, 3' end / clone = RBRCW95 / clone_end = 3' / gb = AI228599 / ug = Rn.3877 / len = 572	295	395	68	42	345	55	0.15942	-6.3
rc_AI236484_at	rc_AI236484 EST233046 Rattus norvegicus cDNA, 3' end / clone = ROVDG74 / clone_end = 3' / gb = AI236484 / ug = Rn.3924 / len = 474	124	115	247	263	119.5	255	2.133891	2.1
rc_H31351_at	rc_H31351 EST105310 Rattus norvegicus cDNA, 3' end / clone = RPCAH85 / clone_end = 3' / gb = H31351 / gi = 976768 / ug = Rn.14564 / len = 352	437	382	265	188	409.5	226.5	0.553114	-1.8
S70803_g_at	S70803 clone p10.15 product [rats, osteosarcoma ROS17/2.8, mRNA, 737 nt]	584	699	147	199	641.5	173	0.26968	-3.7
U01146_s_at	U01146 RRU01146 Rattus rattus Sprague Dawley nuclear orphan receptor HZF-3 (HZF-3) mRNA, complete cds	432	367	586	799	399.5	692.5	1.733417	1.7

Table 5 (cont'd)

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
U14192complete_seq_at	U14192completeSeq Rattus norvegicus general vesicular transport factor p115 mRNA, complete cds / cds = 11,2890 / gb = U14192 / gi = 538152 / ug = Rn.4746 / len = 2891	311	292	163	168	301.5	165.5	0.548922	-1.8
X03347cds_g_at	X03347cds REMSVFBR FBR-murine osteosarcoma provirus genome	232	304	461	496	268	478.5	1.785448	1.8
X12554cds_s_at	X12554cds RNCOX6AH Rat mRNA for heart cytochrome c oxidase subunit Via	269	222	401	470	245.5	435.5	1.773931	1.8
X63446_at	X63446 R.norvegicus mRNA for fetuin / cds = 31,1089 / gb = X63446 / gi = 56139 / ug = Rn.3880 / len = 1456	520	388	248	249	454	248.5	0.547357	-1.8

[142] Genes that passed the filtering criteria outlined above for differential expression between 1 week extinction and 1 week withdrawal in the CeA. Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 6. CeA 1-Week Withdrawal to Control

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
AB003753cds #1_at	AB003753cds#1 Rattus norvegicus genes for high sulfur protein B2E and high sulfur protein B2F, complete cds	373	366	82	126	369.5	104	0.281461	-3.6
AB015433_s_ at	AB015433 Rattus norvegicus mRNA for 4F2 heavy chain (4F2hc), complete cds	269	253	609	524	261	566.5	2.170498	2.2
AB016160_g_ at	AB016160 Rattus norvegicus mRNA for GABAB receptor 1c, complete cds	414	318	148	150	366	149	0.407104	-2.5
AF063302mR NA#3_s_at	AF063302mRNA#3 Rattus norvegicus carnitine palmitoyltransferase Ibeta 1, carnitine palmitoyl-transferase Ibeta 2, and carnitine palmitoyl-transferase Ibeta 3 gene, nuclear gene encoding mitochondrial proteins, alternatively spliced products, partial cds	421	395	140	-4	408	68	0.166667	-6.0
AF064856_at	AF064856 Rattus sp. 7acomp protein mRNA, complete cds	561	529	332	244	545	288	0.52844	-1.9
AF081144_s_ at	AF081144 Rattus norvegicus CL1AA mRNA, complete cds	288	202	495	578	245	536.5	2.189796	2.2
D10853_at	D10853 RATATR Rat mRNA for amidophos-phoribosyltransferase	240	226	119	115	233	117	0.502146	-2.0
D13309_s_at	D13309 RATRDBPB Rat mRNA for DNA-binding protein B	626	625	348	359	625.5	353.5	0.565148	-1.8
D64085_at	D64085 RATORFA1 Rat mRNA for fibroblast growth factor FGF-5, complete cds	443	344	114	245	393.5	179.5	0.456163	-2.2
D83538_g_at	D83538 Rat mRNA for 230kDa phosphatidylinositol 4-kinase, complete cds / cds = 391,6516 / gb = D83538 / gi = 1339965 / ug = Rn.11015 / len = 6857	178	202	386	470	190	428	2.252632	2.3
J00771_at	J00771 RATPRNASE Rat pancreatic ribonuclease mRNA	262	238	-50	-13	250	-31.5	-0.126	7.9
L07398_at	L07398 RATIGVCL Rattus norvegicus (hybridoma 56R-3) immunoglobulin rearranged gamma-chain mRNA variable (V) region, partial cds	305	311	723	611	308	667	2.165584	2.2
L19699_at	L19699 Rat GTP-binding protein (ral B) mRNA, complete cds / cds = 64,684 / gb = L19699 / gi = 310211 / ug = Rn.4586 / len = 2074	331	276	657	711	303.5	684	2.253707	2.3

Table 6 (cont'd)

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
L40364_f_at	L40364 Rattus norvegicus MHC class I RT1.O type - 149 processed pseudogene mRNA / cds = UNKNOWN / gb = L40364 / gi = 992568 / ug = Rn.3577 / len = 1602	177	129	475	403	153	439	2.869281	2.9
M55050_at	M55050 Rattus norwegicus interleukin-2 receptor beta chain (p70/75) mRNA, complete cds / cds = 111,1724 / gb = M55050 / gi = 204913 / ug = Rn.5832 / len = 2598	533	378	231	237	455.5	234	0.513721	-1.9
M81639_at	M81639 Rattus norvegicus stannin mRNA / cds = UNKNOWN / gb = M81639 / gi = 207078 / ug = Rn.6147 / len = 2897	292	316	474	592	304	533	1.753289	1.8
rc_AI639096_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx00904 3', mRNA sequence [Rattus norvegicus]	111	239	392	383	175	387.5	2.214286	2.2
rc_AI639391_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx02754 3', mRNA sequence [Rattus norvegicus]	982	1009	284	334	995.5	309	0.310397	-3.2
rc_AI638980_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx03968 3', mRNA sequence [Rattus norvegicus]	631	601	277	221	616	249	0.404221	-2.5
rc_AI639195_r_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx04881 3', mRNA sequence [Rattus norvegicus]	822	933	519	393	877.5	456	0.519658	-1.9
rc_AA799421_at	rc_AA799421 EST188918 Rattus norvegicus cDNA, 3' end / clone = RHEAA87 / clone_end = 3' / gb = AA799421 / gi = 2862376 / ug = Rn.19951 / len = 570	359	319	479	675	339	577	1.702065	1.7
rc_AA799449_g_at	rc_AA799449 EST188946 Rattus norvegicus cDNA, 3' end / clone = RHEAB19 / clone_end = 3' / gb = AA799449 / gi = 2862404 / ug = Rn.3286 / len = 553	262	327	470	670	294.5	570	1.935484	1.9
rc_AA799671_at	rc_AA799671 EST189168 Rattus norvegicus cDNA, 3' end / clone = RHEAD82 / clone_end = 3' / gb = AA799671 / gi = 2862626 / ug = Rn.6219 / len = 328	421	529	249	297	475	273	0.574737	-1.7
rc_AA799899_i_at	rc_AA799899 EST189396 Rattus norvegicus cDNA, 3' end / clone = RHEAG67 / clone_end = 3' / gb = AA799899 / gi = 2862854 / ug = Rn.5974 / len = 505	4497	3805	6266	7851	4151	7058.5	1.700434	1.7

Table 6 (cont'd)

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
rc_AA859680_g_at	rc_AA859680 UI-R-E0-bs-d-12-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bs-d-12-0-UI / clone_end = 3' / gb = AA859680 / gi = 2949200 / ug = Rn.22632 / len = 437	731	959	2002	1841	845	1921.5	2.273964	2.3
rc_AA875054_at	rc_AA875054 UI-R-E0-cb-e-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cb-e-04-0-UI / clone_end = 3' / gb = AA875054 / gi = 2980002 / ug = Rn.24874 / len = 485	779	581	320	455	680	387.5	0.569853	-1.8
rc_AA891438_g_at	rc_AA891438 EST195241 Rattus norvegicus cDNA, 3' end / clone = RHEAU25 / clone_end = 3' / gb = AA891438 / gi = 3018317 / ug = Rn.22406 / len = 397	557	438	58	235	497.5	146.5	0.294472	-3.4
rc_AA891690_at	rc_AA891690 EST195493 Rattus norvegicus cDNA, 3' end / clone = RKIAF58 / clone_end = 3' / gb=AA891690 / gi = 3018569 / ug = Rn.22701 / len = 446	316	308	167	189	312	178	0.570513	-1.8
rc_AA892859_at	rc_AA892859 EST196662 Rattus norvegicus cDNA, 3' end / clone = RKIAY19 / clone_end = 3' / gb = AA892859 / gi = 3019738 / ug = Rn.8137 / len = 568	236	225	-51	-31	230.5	-41	-0.17787	5.6
rc_AA899106_at	rc_AA899106 UI-R-E0-cw-d-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cw-d-04-0-UI / clone_end = 3' / gb = AA899106 / gi = 3034460 / ug = Rn.6031 / len = 523	550	698	170	185	624	177.5	0.284455	-3.5
rc_AA944422_at	rc_AA944422 EST199921 Rattus norvegicus cDNA, 3' end / clone = REMAJ01 / clone_end = 3' / gb = AA944422 / gi = 3104338 / ug = Rn.871 / len = 641	109	240	382	519	174.5	450.5	2.581662	2.6
rc_AI060085_s_at	rc_AI060085 UI-R-C1-li-c-08-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C1-li-c-08-0-UI / clone_end = 3' / gb = AI060085 / ug = Rn.9967 / len=315	263	258	137	117	260.5	127	0.487524	-2.1
rc_AI138143_at	rc_AI138143 UI-R-C0-if-e-07-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C0-if-e-07-0-UI / clone_end = 3' / gb = AI138143 / ug = Rn.10708 / len = 343	219	210	119	101	214.5	110	0.512821	-2.0
rc_AI170212_s_at	rc_AI170212 EST216137 Rattus norvegicus cDNA, 3' end / clone = RLUCF03 / clone_end = 3' / gb = AI170212 / gi = 3710252 / ug = Rn.11007 / len = 322	271	280	552	626	275.5	589	2.137931	2.1

Table 6 (cont'd)

Probe set no.	Description	1-week extinction	1-week with-drawal	1-week extinction control	1-week with-drawal control	Mean control	Mean extinction	Ratio	Fold change
rc_AI170268_at	rc_AI170268 EST216194 Rattus norvegicus cDNA, 3' end / clone = RLUCG30 / clone_end = 3' / gb = AI170268 / gi = 3710308 / ug = Rn.1868 / len = 577	361	290	492	620	325.5	556	1.708141	1.7
rc_AI176488_at	rc_AI176488 EST220073 Rattus norvegicus cDNA, 3' end / clone = ROVBS47 / clone_end = 3' / gb = AI176488 / ug = Rn.9909 / len = 650	300	391	188	27	345.5	107.5	0.311143	-3.2
rc_AI228599_at	rc_AI228599 EST225294 Rattus norvegicus cDNA, 3' end / clone = RBRCW95 / clone_end = 3' / gb = AI228599 / ug = Rn.3877 / len = 572	-79	-37	295	395	-58	345	-5.94828	at least 2-fold
rc_AI231519_at	rc_AI231519 EST228207 Rattus norvegicus cDNA, 3' end / clone = REMDL26 / clone_end = 3' / gb = AI231519 / ug = Rn.6602 / len = 482	175	180	403	361	177.5	382	2.152113	2.2
Rc_H33651_at	rc_H33651 EST109846 Rattus norvegicus cDNA, 3' end / clone = RPNAV67 / clone_end = 3' / gb = H33651 / gi = 979068 / ug = Rn.14654 / len = 447	406	309	216	189	357.5	202.5	0.566434	-1.8
U14414_at	U14414 Rattus norvegicus P2x receptor mRNA, complete cds / cds = 36,1454 / gb = U14414 / gi = 558830 / ug = Rn.10991 / len = 1831	281	294	126	129	287.5	127.5	0.443478	-2.3
U70270UTR#1_f_at	U70270UTR#1 RNMUD402 Rattus norvegicus mud-4 mRNA, 3' UTR	537	468	-153	66	502.5	-43.5	-0.08657	11.6
U75921UTR#1_at	U75921UTR#1 RNAPCBP3 Rattus norvegicus APC binding protein EB1 mRNA, 3' untranslated region, partial sequence	412	388	122	181	400	151.5	0.37875	-2.6
X03347cds_at	X03347cds REMSVFBR FBR-murine osteosarcoma provirus genome	463	513	252	117	488	184.5	0.378074	-2.6
X12554cds_s_at	X12554cds RNCOX6AH Rat mRNA for heart cytochrome c oxidase subunit VIa	544	449	269	222	496.5	245.5	0.494461	-2.0
X15679_at	X15679 Rat mRNA for preprotrypsinogen IV (EC 3.4.21.4) / cds = 14,757 / gb = X15679 / gi = 56813 / ug = Rn.10387 / len = 862	707	595	371	362	651	366.5	0.56298	-1.8
X60651mRNA_s_at	X60651mRNA RNSYNDCN Rat mRNA for syndecan	407	374	169	191	390.5	180	0.460948	-2.2
X73579_at	X73579 R.norvegicus CD23 mRNA / cds = 0,929 / gb = X73579 / gi = 313672 / ug = Rn.10326 / len = 1146	-43	23	466	604	-10	535	-53.5	at least 2-fold

Genes that passed the filtering criteria outlined above for differential expression between 1 week withdrawal and its corresponding control in the CeA. Average difference values (from

GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 7. Core 1-Week Extinction to Control

Experiment	Description	1-week extinction control core	1-week extinction control core	1-week extinction core	1-week extinction core	Fold change
K02248cds_s_at	K02248cds RATSOM141 Rat somatostatin-14 gene, complete cds	575	528	342	274	-1.8
M55534mRNA_s_at	M55534mRNA Rat alpha-crystallin B chain mRNA, complete cds / cds = UNKNOWN / gb = M55534 / gi = 203609 / ug = Rn.832 / len = 1247	167	264	416	414	1.8
Rc_AA894296_at	rc_AA894296 EST198099 Rattus norvegicus cDNA, 3' end / clone = RSPAW17 / clone_end = 3' / gb = AA894296 / gi = 3021175 / ug = Rn.3760 / len = 600	209	217	436	362	1.9
Rc_AI058941_s_at	rc_AI058941 UI-R-C1-lr-b-07-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C1-lr-b-07-0-UI / clone_end = 3' / gb = AI058941 / ug = Rn.4231 / len = 476	222	-3	389	372	1.8
X15679_at	X15679 Rat mRNA for preprotrypsinogen IV (EC 3.4.21.4) / cds = 14,757 / gb = X15679 / gi = 56813 / ug = Rn.10387 / len = 862	353	365	201	120	-1.8
X95990exon_s_at	X95990exon RNC5ARECP R.norvegicus mRNA for C5a anaphylatoxin receptor	645	544	328	360	-1.7
Z11581_at	Z11581 R.norvegicus mRNA for kainate receptor subunit (ka2) / cds = 202,3141 / gb = Z11581 / gi = 56509 / ug = Rn.10053 / len = 3702	683	724	357	460	-1.7
U05013_at	U05013 Rattus norvegicus Sprague-Dawley heme oxygenase-2 non-reducing isoform gene, complete cds / cds = 177,1124 / gb = U05013 / gi = 501034 / ug = Rn.10241 / len = 1815	209	241	48	53	4.4
M64785_g_at	M64785 RATVAS Rat vasopressin (VP) mRNA	200	211	116	110	1.8

[143] Genes that passed the filtering criteria outlined above for differential expression between 1 week extinction and its corresponding control in the nucleus accumbens core. Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 8. Core 1-Week Extinction to Withdrawal

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
AF055714UTR#1_at	AF055714UTR#1 Rattus norvegicus hypertension-regulated vascular factor-1C-4 mRNA, 3' UTR	481	466	2	-17	-2.4
AF102855_at	AF102855 Rattus norvegicus synaptic SAPAP-interacting protein Synamon mRNA, complete cds	238	264	110	109	2
M11071_f_at	M11071 Rat MHC class I cell surface antigen mRNA / cds = 0,330 / gb = M11071 / gi = 205414 / ug = Rn.11168 / len = 824	1021	897	2204	1642	2.0
M25890_at	M25890 Rat somatostatin mRNA, complete cds / cds = 60,410 / gb = M25890 / gi = 207030 / ug = Rn.540 / len = 564	875	668	1269	1448	1.8
M92076_at	M92076 RATMGLURC Rat metabotropic glutamate receptor 3 Mrna, primary transcript	256	359	709	668	2.2
M95591_g_at	M95591 RATSST Rattus rattus hepatic squalene synthetase mRNA, complete cds	472	494	141	235	-2.2
M96626_g_at	M96626 RAT plasma membrane CA2+-ATPase isoform 3 mRNA, partial cds / cds = 0,346 / gb = M96626 / gi = 203212 / ug = Rn.11053 / len = 609	206	222	96	76	2
rc_AI638989_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx01268 3', mRNA sequence [Rattus norvegicus]	168	135	451	368	2.0
rc_AA819776_f_at	rc_AA819776 UI-R-A0-ap-h-07-0-UI.s1 UI-R-A0 Rattus norvegicus cDNA clone UI-R-A0-ap-h-07-0-UI 3' similar to gb|J04633|MUSHSP86A Mouse heat shock protein 86 mRNA, complete cds, and 28S ribosomal RNA, partial sequence, mRNA sequence [Rattus norvegicus]	56	-42	471	384	2.1
rc_AA858621_g_at	rc_AA858621 UI-R-E0-bq-b-10-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bq-b-10-0-UI / clone_end = 3' / gb = AA858621 / gi = 2948961 / ug = Rn.3551 / len = 550	439	335	691	870	2.0
rc_AA859520_at	rc_AA859520 UI-R-E0-br-b-02-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-br-b-02-0-UI / clone_end = 3' / gb = AA859520 / gi = 2949040 / ug = Rn.23034 / len = 453	230	297	535	507	2.0
rc_AA859966_i_at	rc_AA859966 UI-R-E0-ca-g-03-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-ca-g-03-0-UI / clone_end = 3' / gb = AA859966 / gi = 2949486 / ug = Rn.861 / len = 392	-129	-223	5469	5453	27.3
rc_AA875103_at	rc_AA875103 UI-R-E0-cf-h-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cf-h-04-0-UI / clone_end = 3' / gb = AA875103 / gi = 2980051 / ug = Rn.22643 / len = 606	299	266	-20	-49	14
rc_AA875131_at	rc_AA875131 UI-R-E0-bu-e-03-0-UI.s2 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bu-e-03-0-UI / clone_end = 3' / gb = AA875131 / gi = 2980079 / ug = Rn.2801 / len = 575	381	429	186	231	-1.9

Table 8 (cont'd)

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
rc_AA891721_at	rc_AA891721 EST195524 Rattus norvegicus cDNA, 3' end / clone = RKIAF94 / clone_end = 3' / gb = AA891721 / gi = 3018600 / ug = Rn.14709 / len = 454	342	417	166	170	-1.9
rc_AA893065_at	rc_AA893065 EST196868 Rattus norvegicus cDNA, 3' end / clone = RKIBB69 / clone_end = 3' / gb = AA893065 / gi = 3019944 / ug = Rn.13472 / len = 410	225	254	516	489	2.1
rc_AA893612_at	rc_AA893612 EST197415 Rattus norvegicus cDNA, 3' end / clone = RPLAC57 / clone_end = 3' / gb = AA893612 / gi = 3020491 / ug = Rn.14814 / len = 265	517	514	942	919	1.8
rc_AA893870_g_at	rc_AA893870 EST197673 Rattus norvegicus cDNA, 3' end / clone = RPLAM86 / clone_end = 3' / gb = AA893870 / gi = 3020749 / ug = Rn.11229 / len = 417	46	62	308	316	6
rc_AA945054_s_at	rc_AA945054 EST200553 Rattus norvegicus cDNA, 3' end / clone = RLIAF82 / clone_end = 3' / gb = AA945054 / ug = Rn.1055 / len = 565	449	573	801	975	1.7
rc_AA955983_at	rc_AA955983 UI-R-E1-fb-e-12-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E1-fb-e-12-0-UI / clone_end = 3' / gb = AA955983 / ug = Rn.7854 / len = 542	579	704	351	398	-1.7
rc_AI008863_at	rc_AI008863 EST203314 Rattus norvegicus cDNA, 3' end / clone = REMBE50 / clone_end = 3' / gb = AI008863 / ug = Rn.1893 / len = 401	322	450	196	245	-1.7
rc_AI013194_at	rc_AI013194 EST207869 Rattus norvegicus cDNA, 3' end / clone = RSPBH90 / clone_end = 3' / gb = AI013194 / ug = Rn.3506 / len = 464	217	251	584	531	2
rc_AI014135_g_at	rc_AI014135 EST207690 Rattus norvegicus cDNA, 3' end / clone = RSPBF48 / clone_end = 3' / gb = AI014135 / ug = Rn.4229 / len = 410	1499	1401	567	444	3
rc_AI102103_at	rc_AI102103 EST211392 Rattus norvegicus cDNA, 3' end / clone = RBRBY91 / clone_end = 3' / gb = AI102103 / gi = 3706936 / ug = Rn.14991 / len = 611	1193	1211	698	655	-1.8
rc_AI172097_g_at	rc_AI172097 EST218092 Rattus norvegicus cDNA, 3' end / clone = RMUBU88 / clone_end = 3' / gb = AI172097 / gi = 3712137 / ug = Rn.20418 / len = 570	274	323	541	556	1.8
rc_H31982_at	rc_H31982 EST106584 Rattus norvegicus cDNA, 3' end / clone = RPCBE17 / clone_end = 3' / gb = H31982 / gi = 977399 / ug = Rn.7138 / len = 363	354	431	170	175	-2.0
U62897_at	U62897 Rattus norvegicus carboxypeptidase D precursor (Cpd) mRNA, complete cds / cds = 45,4181 / gb=U62897 / gi = 2406562 / ug = Rn.4093 / len = 4377	183	216	344	435	1.9
U67995_s_at	U67995 Rattus norvegicus stearyl-CoA desaturase 2 mRNA, partial cds / cds = 0,92 / gb = U67995 / gi = 1763026 / ug = Rn.10650 / len = 315	1336	1291	780	591	-1.9

Table 8 (cont'd)

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
U77931_at	U77931 RNU77931 Rattus norvegicus unknown mRNA	836	912	2166	1850	2.3
X05472cds#2_at	X05472cds#2 RNREP24R Rat 2.4 kb repeat DNA right terminal region	4218	4342	945	541	6
X06564_at	X06564 Rat mRNA for 140-kD NCAM polypeptide / cds = 208,2784 / gb = X06564 / gi = 56736 / ug = Rn.11283 / len = 3170	47	28	309	281	8
X12744_at	X12744 Rat mRNA for c-erb-A thyroid hormone receptor / cds = 0,1198 / gb = X12744 / gi = 55931 / ug = Rn.11307 / len = 1775	255	252	499	442	1.9
X15679_at	X15679 Rat mRNA for preprotrypsinogen IV (EC 3.4.21.4) / cds = 14,757 / gb = X15679 / gi = 56813 / ug = Rn.10387 / len = 862	377	403	120	201	-1.9
X70667cds_at	X70667cds RRMC3RA R.rattus mRNA for melanocortin-3 receptor	221	249	426	508	2.0
AFFX_rat_5S_rRNA_at	X83747 Rattus norvegicus 5S rRNA gene (clone pRA5S2).	348	357	154	146	2

[144] Genes that passed the filtering criteria outlined above for differential expression between 1 week extinction and 1 week withdrawal in the nucleus accumbens core. Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 9. Core 1-Week Withdrawal to Control

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
AB008424_s_at	AB008424 Rattus norvegicus mRNA for CYP2D3, complete cds	376	453	180	153	-2.1
AF069525_at	AF069525 Rattus norvegicus 190 kDa ankyrin isoform mRNA, complete cds / cds = 84,5372 / gb = AF069525 / gi = 3202045 / ug = Rn.236 / len = 6184	275	234	504	424	1.8
AF077354_g_at	AF077354 Rattus norvegicus ischemia responsive 94 kDa protein (irp94) mRNA, complete cds	61	81	244	251	3.5
AJ005425_at	AJ005425 RNAJ5425 Rattus norvegicus mRNA for MEF2D protein	86	22	373	394	1.9
L07398_at	L07398 RATIGVCL Rattus norvegicus (hybridoma 56R-3) immunoglobulin rearranged gamma-chain mRNA variable (V) region, partial cds	437	308	231	156	-1.7
M80826_at	M80826 Rat intestinal trefoil protein mRNA, complete cds / cds = 17,262 / gb = M80826 / gi = 207446 / ug = Rn.9960 / len = 431	334	322	112	102	3.1
Rc_AI639392_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx02714 3', mRNA sequence [Rattus norvegicus]	291	393	96	89	-1.7
Rc_AA875131_at	rc_AA875131 UI-R-E0-bu-e-03-0-UI.s2 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bu-e-03-0-UI / clone_end = 3' / gb = AA875131 / gi = 2980079 / ug = Rn.2801 / len = 575	201	260	429	381	1.8
Rc_AA899106_at	rc_AA899106 UI-R-E0-cw-d-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cw-d-04-0-UI / clone_end = 3' / gb = AA899106 / gi = 3034460 / ug = Rn.6031 / len = 523	105	120	252	273	2.3
Rc_AI230778_at	rc_AI230778 EST227473 Rattus norvegicus cDNA, 3' end / clone = REMDB16 / clone_end = 3' / gb = AI230778 / ug = Rn.3659 / len = 560	341	359	142	122	-1.8
Rc_AI230778_at	rc_AI230778 EST227473 Rattus norvegicus cDNA, 3' end / clone = REMDB16 / clone_end = 3' / gb = AI230778 / ug = Rn.3659 / len = 560	359	341	122	142	2.7
U38180_at	U38180 Rattus norvegicus reduced folate carrier membrane glycoprotein mRNA, complete cds / cds = 248,1786 / gb = U38180 / gi = 1022954 / ug = Rn.9042 / len = 2410	124	110	277	253	2.3
U70268UTR#1_at	U70268UTR#1 RNMUD702 Rattus norvegicus mud-7 mRNA, 3' UTR	670	600	317	363	-1.9
X56729mRNA_at	X56729mRNA RSCALPST Rat mRNA for calpastatin	324	322	64	64	5.1

Genes that passed the filtering criteria outlined above for differential expression between 1 week withdrawal and its corresponding control in the nucleus accumbens core. Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 10. mPFC F Id Change 1-Week Extinction to Control

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
AB002393_at	AB002393 Rattus norvegicus mRNA for histidase, partial cds	230	198	-38	-45	-10.7
AB012234_g_at	AB012234 Rattus norvegicus mRNA for NF1-X1, partial cds / cds = 0,535 / gb = AB012234 / gi = 2982735 / ug = Rn.9647 / len = 601	719	751	440	358	-1.8
AF050663UTR#1_at	AF050663UTR#1 Rattus norvegicus activity and neurotransmitter-induced early gene 11 (ania-11) mRNA, 3' UTR	492	471	200	173	-2.6
AF081204_s_at	AF081204 Rattus norvegicus small intestine sodium dependent multivitamin transporter (SMVT) mRNA, complete cds	414	402	220	212	-1.9
AF102854_at	AF102854 Rattus norvegicus membrane-associated guanylate kinase-interacting protein 2 Maguin-2 mRNA, complete cds	458	430	190	123	-2.2
AJ005113_g_at	AJ005113 RNAJ5113 Rattus norvegicus mRNA for SMC-protein Molecular characterization of a rat heterochromatin associated SMC-protein	447	469	232	258	-1.9
AJ011115_at	AJ011115 RNO011115 Rattus norvegicus mRNA for endothelial nitric oxide synthase, 5' region, partial	425	315	83	129	-1.9
AJ012603UTR#1_at	AJ012603UTR#1 RNO012603 Rattus norvegicus mRNA for TNF-alpha converting enzyme (TACE)	520	442	211	237	-2.1
D00512_g_at	D00512 RATACAL Rattus sp. mRNA for mitochondrial acetoacetyl-CoA thiolase precursor, complete cds	464	365	203	173	-2.1
D30040_at	D30040 Rat mRNA for RAC protein kinase alpha, complete cds / cds = 42,1484 / gb = D30040 / gi = 485402 / ug = Rn.11422 / len = 1617	206	229	383	450	1.9
E01415cds_s_at	E01415cds cDNA encoding rat glutathione S transferase	975	687	501	460	-1.7
J02592_s_at	J02592 Rat glutathione S-transferase Y-b subunit mRNA, 3' end / cds = 0,560 / gb = J02592 / gi = 204498 / ug = Rn.625 / len = 909	1022	746	265	347	-2.9
J05155_at	J05155 Rat phospholipase C type IV mRNA, complete cds / cds = 200,3997 / gb = J05155 / gi = 206242 / ug = Rn.9751 / len = 4321	228	222	72	88	-2.8
K01701_at	K01701 Rat oxytocin/neurophysin (Oxt) gene, complete gene, complete cds / cds = 41,418 / gb = K01701 / gi = 205899 / ug = Rn.11315 / len = 530	150	162	418	508	2.3
L37971mRNA_at	L37971mRNA RATTCRAP Rattus norvegicus T-cell receptor alpha-chain mRNA	349	340	171	203	-1.7
L38482_at	L38482 Rattus norvegicus serine protease gene, complete cds / cds = 0,401 / gb = L38482 / gi = 1020080 / ug = Rn.2427 / len = 402	253	355	687	603	2.1

Table 10 (cont'd)

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
M22756_at	M22756 Rat 24-kDa subunit of mitochondrial NADH dehydrogenase mRNA, 3' end / cds = 0,725 / gb = M22756 /gi = 205627 / ug = Rn.11092 / len = 771	1247	1029	604	651	-1.8
M25804_g_at	M25804 Rat Rev-ErbA-alpha protein mRNA, complete cds / cds = 501,2027 / gb = M25804 / gi = 514963 / ug = Rn.10105 / len = 2297	58	161	418	365	2.0
M27886exon_g_at	M27886exon RAT6PF2KFR Rattus norvegicus bifunctional enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (6-PF2-K/ Fru-2,6-P-2-ase) gene, exon 1	308	301	72	72	-4.2
M31032cds#1_s_at	M31032cds#1 RATCRP01 Rat contiguous repeat polypeptides (CRP) mRNA, complete cds	426	354	180	176	-2.0
M32061_at	M32061 Rat alpha-2B-adrenergic receptor (RNG-alpha-2) mRNA, complete cds / cds = 365,1726 / gb = M32061 / gi = 202589 / ug = Rn.10296 / len = 2319	158	236	508	481	2.3
M76535cds_at	M76535cds RATCXN40A Rat gap junction structural protein, connexin (CXN-40) gene, complete cds	734	746	353	278	-2.4
M77245_at	M77245 R.norvegicus beta'-chain clathrin associated protein complex AP-1 mRNA, complete cds / cds = 39,2888 / gb = M77245 / gi = 203112 / ug = Rn.9466 / len = 3663	23	162	415	512	2.3
M77246_at	M77246 R.norvegicus beta-chain clathrin associated protein complex AP-2 mRNA, complete cds / cds = 139,2994 / gb = M77246 / gi = 203114 / ug = Rn.1050 / len = 5402	585	580	1166	1215	2.0
M97662_at	M97662 Rattus norvegicus beta-alanine synthase mRNA, complete cds / cds = 33,1214 / gb = M97662 / gi = 203105 / ug = Rn.11110 / len = 1420	406	425	204	140	-2.1
rc_AI639272_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx03958 3', mRNA sequence [Rattus norvegicus]	248	261	55	73	-4.0
rc_AI639313_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx04777 3', mRNA sequence [Rattus norvegicus]	576	564	191	154	-3.3
rc_AI639195_r_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx04881 3', mRNA sequence [Rattus norvegicus]	-73	84	847	1043	4.7
rc_AA684641_at	rc_AA684641 EST104995 Rattus norvegicus cDNA, 3' end / clone = RPCAE71 / clone_end = 3' / gb = AA684641 / gi = 2671239 / ug = Rn.14675 / len = 249	135	197	356	348	1.8
rc_AA799525_at	rc_AA799525 EST189022 Rattus norvegicus cDNA, 3' end / clone = RHEAC13 / clone_end = 3' / gb = AA799525 / gi = 2862480 / ug = Rn.1099 / len = 573	682	583	371	370	-1.7
rc_AA799531_g_at	rc_AA799531 EST189028 Rattus norvegicus cDNA, 3' end / clone = RHEAC22 / clone_end = 3' / gb = AA799531 / gi = 2862486 / ug = Rn.6198 / len = 570	586	471	247	329	-1.8

Table 10 (cont'd)

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
rc_AA818152_f_at	rc_AA818152 UI-R-A0-am-b-09-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-A0-am-b-09-0-UI / clone_end = 3' / gb = AA818152 / gi = 2888032 / ug = Rn.16465 / len = 117	6678	7495	3932	4179	-1.7
rc_AA818226_s_at	rc_AA818226 UI-R-A0-ah-g-06-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-A0-ah-g-06-0-UI / clone_end = 3' / gb = AA818226 / gi = 2888106 / ug = Rn.2528 / len = 609	5530	4813	2582	3212	-1.8
rc_AA851403_g_at	rc_AA851403 EST194171 Rattus norvegicus cDNA, 3' end / clone = RPLAG17 / clone_end = 3' / gb = AA851403 / gi = 2938943 / ug = Rn.3383 / len = 393	1728	1725	781	1074	-1.9
rc_AA851403_at	rc_AA851403 EST194171 Rattus norvegicus cDNA, 3' end / clone = RPLAG17 / clone_end = 3' / gb = AA851403 / gi = 2938943 / ug = Rn.3383 / len = 393	291	296	131	127	-2.3
rc_AA852004_s_at	rc_AA852004 EST194773 Rattus norvegicus cDNA, 3' end / clone = RSPAP38 / clone_end = 3' / gb = AA852004 / gi = 2939544 / ug = Rn.2204 / len = 368	780	722	1393	1184	1.7
rc_AA859299_at	rc_AA859299 UI-R-E0-cj-b-02-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cj-b-02-0-UI / clone_end = 3' / gb = AA859299 / gi = 2948650 / ug = Rn.9517 / len = 529	309	302	721	553	2.1
rc_AA859837_g_at	rc_AA859837 UI-R-E0-cc-g-09-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cc-g-09-0-UI / clone_end = 3' / gb = AA859837 / gi = 2949357 / ug = Rn.24783 / len = 486	3301	2600	1719	1534	-1.8
rc_AA859922_at	rc_AA859922 UI-R-E0-cg-c-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cg-c-04-0-UI / clone_end = 3' / gb = AA859922 / gi = 2949442 / ug = Rn.819 / len = 373	657	601	253	321	-2.2
rc_AA866477_at	rc_AA866477 UI-R-E0-br-h-03-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-br-h-03-0-UI / clone_end = 3' / gb = AA866477 / gi = 2961938 / ug = Rn.2026 / len = 488	1136	1364	601	702	-1.9
rc_AA875420_at	rc_AA875420 UI-R-E0-cs-e-08-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cs-e-08-0-UI / clone_end=3' / gb = AA875420 / gi = 2980368 / ug = Rn.21413 / len = 499	291	339	20	47	-9.4
rc_AA892006_at	rc_AA892006 EST195809 Rattus norvegicus cDNA, 3' end / clone = RKIAK60 / clone_end = 3' / gb = AA892006 / gi = 3018885 / ug = Rn.11519 / len = 443	-157	-160	510	449	23.96
rc_AA892800_at	rc_AA892800 EST196603 Rattus norvegicus cDNA, 3' end / clone = RKIAX43 / clone_end = 3' / gb = AA892800 / gi = 3019679 / ug = Rn.3609 / len = 493	-203	-165	390	313	1.8
rc_AA894296_at	rc_AA894296 EST198099 Rattus norvegicus cDNA, 3' end / clone = RSPAW17 / clone_end = 3' / gb = AA894296 / gi = 3021175 / ug = Rn.3760 / len = 600	222	252	457	573	2.2

Table 10 (cont'd)

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
rc_AA899106_at	rc_AA899106 UI-R-E0-cw-d-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cw-d-04-0-UI / clone_end = 3' / gb = AA899106 / gi = 3034460 / ug = Rn.6031 / len = 523	482	459	292	214	-1.9
rc_AA899253_at	rc_AA899253 UI-R-E0-cz-g-07-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cz-g-07-0-UI / clone_end = 3' / gb = AA899253 / gi = 3034607 / ug = Rn.9560 / len = 410	832	904	401	537	-1.9
rc_AA945152_s_at	rc_AA945152 EST200651 Rattus norvegicus cDNA, 3' end / clone = RLIAH24 / clone_end = 3' / gb = AA945152 / ug = Rn.4241 / len = 777	22042	30447	12827	15228	-1.9
rc_AI009191_at	rc_AI009191 EST203642 Rattus norvegicus cDNA, 3' end / clone = REMBK67 / clone_end = 3' / gb = AI009191 / ug = Rn.2432 / len = 484	441	615	981	821	1.7
rc_AI058941_s_at	rc_AI058941 UI-R-C1-lr-b-07-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C1-lr-b-07-0-UI / clone_end = 3' / gb = AI058941 / ug = Rn.4231 / len = 476	570	562	214	252	-2.4
rc_AI072770_s_at	rc_AI072770 UI-R-Y0-md-g-02-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-Y0-md-g-02-0-UI / clone_end = 3' / gb = AI072770 / ug = Rn.4550 / len = 333	330	258	462	566	1.7
rc_AI103396_g_at	rc_AI103396 EST212685 Rattus norvegicus cDNA, 3' end / clone = REMCB47 / clone_end = 3' / gb = AI103396 / gi = 3707945 / ug = Rn.221 / len = 443	26045	26104	16586	12308	-1.8
rc_AI137043_at	rc_AI137043 UI-R-C2p-oj-c-01-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C2p-oj-c-01-0-UI / clone_end = 3' / gb = AI137043 / ug = Rn.22168 / len = 436	371	442	95	35	-2.0
rc_AI137856_s_at	rc_AI137856 UI-R-C0-ik-a-10-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C0-ik-a-10-0-UI / clone_end = 3' / gb = AI137856 / ug = Rn.11359 / len = 384	510	469	212	219	-2.3
rc_AI176307_at	rc_AI176307 EST219889 Rattus norvegicus cDNA, 3' end / clone = ROVBP82 / clone_end = 3' / gb = AI176307 / ug = Rn.10427 / len = 678	1777	2176	952	906	-2.1
rc_AI176621_at	rc_AI176621 EST220210 Rattus norvegicus cDNA, 3' end / clone = ROVBU65 / clone_end = 3' / gb = AI176621 / ug = Rn.1979 / len = 620	400	289	196	117	-1.7
rc_AI177503_at	rc_AI177503 EST221135 Rattus norvegicus cDNA, 3' end / clone = RPLCA81 / clone_end = 3' / gb = AI177503 / ug = Rn.11066 / len = 575	276	273	520	454	1.8
rc_AI232012_at	rc_AI232012 EST228700 Rattus norvegicus cDNA, 3' end / clone = RHECR46 / clone_end = 3' / gb = AI232012 / ug = Rn.1128 / len = 586	1062	957	575	590	-1.7
rc_AI232321_at	rc_AI232321 EST229009 Rattus norvegicus cDNA, 3' end / clone = RKICA22 / clone_end = 3' / gb = AI232321 / ug = Rn.24630 / len = 590	312	333	177	173	-1.8

Table 10 (cont'd)

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
rc_AI234060_s_at	rc_AI234060 EST230748 Rattus norvegicus cDNA, 3' end / clone = RLUCU63 / clone_end = 3' / gb = AI234060 / ug = Rn.11372 / len = 363	119	111	322	302	2.71
S74801_s_at	S74801 H(+)-K(+)-ATPase alpha-subunit [rats, Sprague-Dawley, kidney, mRNA Partial, 1361 nt]	238	239	101	78	-2.7
U16025_at	U16025 Rattus norvegicus class Ib RT1 mRNA, complete cds / cds = 0,1019 / gb = U16025 / gi = 717092 / ug = Rn.19044 / len = 1311	470	442	267	211	-1.9
U23769_at	U23769 Rattus norvegicus CLP36 (clp36) mRNA, complete cds / cds = 66,1049 / gb = U23769 / gi = 1020150 / ug = Rn.11170 / len = 1392	172	162	285	284	1.7
U32575_g_at	U32575 RNU32575 Rattus norvegicus (rsec6) mRNA, complete cds	364	360	32	38	-1.8
U56261_s_at	U56261 RNU56261 Rattus norvegicus SNAP-25a mRNA, partial cds	122	144	300	303	2.27
U70270UTR#1_f_at	U70270UTR#1 RNMUD402 Rattus norvegicus mud-4 mRNA, 3' UTR	550	516	340	270	-1.7
U72995_at	U72995 Rattus norvegicus Rab3 GDP/GTP exchange protein mRNA, complete cds / cds = 191,4999 / gb = U72995 / gi =1947049 / ug = Rn.9786 / len = 5249	273	248	579	497	2.1
U89745_at	U89745 Rattus norvegicus unknown protein mRNA, partial cds / cds = 0,293 / gb = U89745 / gi = 1895082 / ug = Rn.10720 / len = 1114	1075	1106	654	587	-1.8
X53581cds#5_f_at	X53581cds#5 RNLINED R.norvegicus long interspersed repetitive DNA containing 7 ORF's	1225	1155	2071	2773	2.0
X69903_at	X69903 R.norvegicus mRNA for interleukin 4 receptor / cds = 9,2411 / gb = X69903 / gi = 56390 / ug = Rn.10471 / len = 2450	491	377	118	146	-2.2
Y17048_g_at	Y17048 RNCALDE Rattus norvegicus mRNA for caldendrin	492	465	912	916	1.91
Z50052_at	Z50052 R.norvegicus mRNA for C4BP beta chain protein / cds = 265,1041 / gb = Z50052 / gi = 899381 / ug = Rn.11151 / len = 1091	220	226	45	61	-4.2

[145] Genes that passed the filtering criteria outlined above for differential expression between 1 week extinction and its corresponding control in the medial prefrontal cortex (mPFC). Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 11. mPFC Fold Change 1-Week Extinction to Withdrawal

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
AB004559_at	AB004559 Rattus norvegicus mRNA for multispecific organic anion transporter, complete cds / cds = 275,1930 / gb = AB004559 / gi = 2361034 / ug = Rn.11113 / len = 2221	34	109	393	431	-2.1
AF020618_g_at	AF020618 Rattus norvegicus progression elevated gene 3 protein mRNA, complete cds	371	416	205	139	1.9
AF044201_at	AF044201 Rattus norvegicus neural membrane protein 35 mRNA, complete cds	1152	974	636	546	1.8
AF051526_at	AF051526 Rattus norvegicus class A calcium channel variant riA-I (BCCA1) mRNA, partial cds / cds = 0,2375 / gb = AF051526 / gi = 2961609 / ug = Rn.11281 / len = 2427	249	242	98	119	-2.3
AF076183_at	AF076183 Rattus norvegicus cytosolic sorting protein PACS-1a (PACS-1) mRNA, complete cds	405	319	215	177	1.7
AF091566_f_at	AF091566 Rattus norvegicus isolate HTF-SP1 olfactory receptor mRNA, partial cds	303	407	9	-44	1.8
AF102854_at	AF102854 Rattus norvegicus membrane-associated guanylate kinase-interacting protein 2 Maguin-2 mRNA, complete cds	379	395	123	190	1.9
AFFX_Rat_beta-actin_5_at	V01217 Rat gene encoding cytoplasmic beta-actin (_5, _M, _3 represent transcript regions 5 prime, Middle, and 3 prime respectively)	1708	2336	992	1100	1.9
AJ005113_at	AJ005113 RNAJ5113 Rattus norvegicus mRNA for SMC-protein Molecular characterization of a rat heterochromatin associated SMC-protein	299	384	103	195	1.7
AJ005394_at	AJ005394 RNJ005394 Rattus norvegicus mRNA for collagen alpha 1 type V	355	309	103	96	-3.3
AJ011005_at	AJ011005 RNO011005 Rattus norvegicus mRNA for Ptx3 protein	848	988	460	359	2.2
D00512_g_at	D00512 RATACAL Rattus sp. mRNA for mitochondrial acetoacetyl-CoA thiolase precursor, complete cds	386	470	173	203	2.1
D10757_at	D10757 RATPRORR12 Rat mRNA for proteasome subunit R-RING12, complete cds	444	484	230	246	-2.0
D13212_s_at	D13212 RATNMDARC Rat mRNA for N-methyl-D-aspartate receptor subunit (NMDAR2C)	484	499	251	280	-1.9
D14819_g_at	D14819 RATCBPP23B Rat mRNA for calcium-binding protein P23k beta, partial cds	679	890	484	392	1.8
D30734_at	D30734 RATGAP1M Rat mRNA for Ras GTPase-activating protein, complete cds	353	383	220	208	1.7

Table 11 (cont'd)

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
J02669_s_at	J02669 Rat cytochrome P-450a (3-methylchlanthrene-inducible; with high testosterone 7-alpha activity), mRNA, complete cds / cds = 19,1497 / gb = J02669 / gi = 203766 / ug = Rn.10904 / len = 1687	858	1000	539	530	1.7
J05499_at	J05499 Rattus norvegicus L-glutamine amidohydrolase mRNA, complete cds / cds = 131,1738 / gb = J05499 / gi = 1196813 / ug = Rn.10202 / len = 2225	216	215	114	128	-1.8
K01701_at	K01701 Rat oxytocin/neurophysin (Oxt) gene, complete gene, complete cds / cds = 41,418 / gb =K01701 / gi = 205899 / ug = Rn.11315 / len = 530	150	131	508	418	-2.3
L07398_at	L07398 RATIGVCL Rattus norvegicus (hybridoma 56R-3) immunoglobulin rearranged gamma-chain mRNA variable (V) region, partial cds	189	148	449	527	-2.4
L38482_at	L38482 Rattus norvegicus serine protease gene, complete cds / cds = 0,401 / gb = L38482 / gi = 1020080 / ug = Rn.2427 / len = 402	290	360	603	687	-2.0
M11071_f_at	M11071 Rat MHC class I cell surface antigen mRNA / cds = 0,330 / gb = M11071 / gi = 205414 / ug = Rn.11168 / len = 824	3217	3367	792	959	-3.8
M20721_f_at	M20721 RATPRPA Rat proline-rich protein (PRP-1) mRNA, partial cds	282	281	129	128	-2.2
M25804_g_at	M25804 Rat Rev-ErbA-alpha protein mRNA, complete cds / cds = 501,2027 / gb = M25804 / gi = 514963 / ug = Rn.10105 / len = 2297	175	138	365	418	-2.0
M27886exon_g_at	M27886exon RAT6PF2KFR Rattus norvegicus bifunctional enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (6-PF2-K/ Fru-2,6-P-2-ase) gene, exon 1	223	215	72	72	-3.0
M31018_f_at	M31018 Rattus norvegicus MHC class I RT1.Aa alpha-chain precursor mRNA, complete cds / cds = 9,1124 / gb = M31018 / gi = 1877415 / ug = Rn.3577 / len = 1590	474	454	232	156	2.1
M77809_at	M77809 Rat betaglycan mRNA, complete cds / cds = 334,2895 / gb = M77809 / gi = 203137 / ug = Rn.9953 / len = 3931	378	339	117	120	-3.0
Rc_AA799467_at	rc_AA799467 EST188964 Rattus norvegicus cDNA, 3' end / clone = RHEAB38 / clone_end = 3' / gb = AA799467 / gi = 2862422 / ug = Rn.4036 / len = 568	413	486	292	218	1.8
Rc_AA799792_at	rc_AA799792 EST189289 Rattus norvegicus cDNA, 3' end / clone = RHEAF41 / clone_end = 3' / gb = AA799792 / gi = 2862747 / ug = Rn.7461 / len = 615	101	92	261	291	2.9
Rc_AA799964_at	rc_AA799964 EST189461 Rattus norvegicus cDNA, 3' end / clone = RHEAH66 / clone_end = 3' / gb = AA799964 / gi = 2862919 / ug = Rn.6261 / len = 452	17	3	309	270	14.5
Rc_AA800005_at	rc_AA800005 EST189502 Rattus norvegicus cDNA, 3' end / clone = RHEAI20 / clone_end = 3' / gb = AA800005 / gi = 2862960 / ug = Rn.1465 / len = 628	328	315	701	636	2.1

Table 11 (cont'd)

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
Rc_AA800250_at	rc_AA800250 EST189747 Rattus norvegicus cDNA, 3' end / clone = RHEAM94 / clone_end = 3' / gb = AA800250 / gi = 2863205 / ug = Rn.3593 / len = 666	708	567	912	1264	-1.7
Rc_AA800604_g_at	rc_AA800604 EST190101 Rattus norvegicus cDNA, 3' end / clone = RLUAB65 / clone_end = 3' / gb = AA800604 / gi = 2863559 / ug = Rn.8590 / len = 579	413	396	159	-18	2.0
Rc_AA800737_at	rc_AA800737 EST190234 Rattus norvegicus cDNA, 3' end / clone = RLUAK84 / clone_end = 3' / gb = AA800737 / gi = 2863692 / ug = Rn.6628 / len = 626	219	206	430	322	-1.8
Rc_AA851403_at	rc_AA851403 EST194171 Rattus norvegicus cDNA, 3' end / clone = RPLAG17 / clone_end = 3' / gb = AA851403 / gi = 2938943 / ug = Rn.3383 / len = 393	309	328	131	127	-2.5
Rc_AA859585_at	rc_AA859585 UI-R-E0-bv-d-05-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bv-d-05-0-UI / clone_end = 3' / gb = AA859585 / gi = 2949105 / ug = Rn.24950 / len = 516	471	544	176	262	2.2
Rc_AA859722_at	rc_AA859722 UI-R-E0-bx-h-09-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bx-h-09-0-UI / clone_end = 3' / gb = AA859722 / gi = 2949242 / ug = Rn.70 / len = 460	459	381	-1	5	-21.0
Rc_AA859922_at	rc_AA859922 UI-R-E0-cg-c-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cg-c-04-0-UI / clone_end = 3' / gb = AA859922 / gi = 2949442 / ug = Rn.819 / len = 373	615	712	321	253	2.3
Rc_AA874919_at	rc_AA874919 UI-R-E0-ck-g-09-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-ck-g-09-0-UI / clone_end = 3' / gb = AA874919 / gi = 2979867 / ug = Rn.3174 / len = 542	224	221	365	428	-1.8
Rc_AA875411_s_at	rc_AA875411 UI-R-E0-cs-b-11-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cs-b-11-0-UI / clone_end = 3' / gb = AA875411 / gi = 2980359 / ug = Rn.2911 / len = 423	115	191	425	422	-2.1
Rc_AA892006_at	rc_AA892006 EST195809 Rattus norvegicus cDNA, 3' end / clone = RKIAK60 / clone_end = 3' / gb = AA892006 / gi = 3018885 / ug = Rn.11519 / len = 443	-59	-76	510	449	24.0
Rc_AA892179_at	rc_AA892179 EST195982 Rattus norvegicus cDNA, 3' end / clone = RKIAN31 / clone_end = 3' / gb = AA892179 / gi = 3019058 / ug = Rn.9031 / len = 428	210	198	421	358	-1.9
Rc_AA892800_at	rc_AA892800 EST196603 Rattus norvegicus cDNA, 3' end / clone = RKIAX43 / clone_end = 3' / gb = AA892800 / gi = 3019679 / ug = Rn.3609 / len = 493	35	-350	313	390	-1.8
Rc_AA892801_g_at	rc_AA892801 EST196604 Rattus norvegicus cDNA, 3' end / clone = RKIAX44 / clone_end = 3' / gb = AA892801 / gi = 3019680 / ug = Rn.3610 / len = 528	497	658	277	354	1.8

Table 11 (cont'd)

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
Rc_AA892828_at	rc_AA892828 EST196631 Rattus norvegicus cDNA, 3' end / clone = RKIAX75 / clone_end = 3' / gb = AA892828 / gi = 3019707 / ug = Rn.2273 / len = 626	343	240	444	551	-1.7
Rc_AA893210_at	rc_AA893210 EST197013 Rattus norvegicus cDNA, 3' end / clone = RKIBD55 / clone_end = 3' / gb = AA893210 / gi = 3020089 / ug = Rn.11141 / len = 608	-20	28	329	361	17.3
Rc_AI009191_at	rc_AI009191 EST203642 Rattus norvegicus cDNA, 3' end / clone = REMBK67 / clone_end = 3' / gb = AI009191 / ug = Rn.2432 / len = 484	512	542	821	981	-1.7
Rc_AI013993_at	rc_AI013993 EST207548 Rattus norvegicus cDNA, 3' end / clone = RSPBC95 / clone_end = 3' / gb = AI013993 / ug = Rn.221 / len = 514	279	248	100	102	-2.6
Rc_AI014094_g_at	rc_AI014094 EST207649 Rattus norvegicus cDNA, 3' end / clone = RSPBE87 / clone_end = 3' / gb = AI014094 / ug = Rn.221 / len = 569	374	335	195	187	1.8
Rc_AI101320_at	rc_AI101320 EST210609 Rattus norvegicus cDNA, 3' end / clone = RBRBL38 / clone_end = 3' / gb = AI101320 / ug = Rn.22459 / len = 616	368	341	119	125	-2.9
Rc_AI137856_s_at	rc_AI137856 UI-R-C0-ik-a-10-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C0-ik-a-10-0-UI / clone_end = 3' / gb = AI137856 / ug = Rn.11359 / len = 384	394	392	212	219	-1.8
Rc_AI172097_g_at	rc_AI172097 EST218092 Rattus norvegicus cDNA, 3' end / clone = RMUBU88 / clone_end = 3' / gb = AI172097 / gi = 3712137 / ug = Rn.20418 / len = 570	533	441	123	200	2.4
Rc_AI176307_at	rc_AI176307 EST219889 Rattus norvegicus cDNA, 3' end / clone = ROVBP82 / clone_end = 3' / gb = AI176307 / ug = Rn.10427 / len = 678	1840	1824	906	952	-2.0
Rc_AI231213_g_at	rc_AI231213 EST227901 Rattus norvegicus cDNA, 3' end / clone = REMDH23 / clone_end = 3' / gb = AI231213 / ug = Rn.3022 / len = 582	70	45	213	216	3.7
Rc_AI231472_s_at	rc_AI231472 EST228160 Rattus norvegicus cDNA, 3' end / clone = REMDK57 / clone_end = 3' / gb = AI231472 / ug = Rn.2953 / len = 549	160	171	384	349	2.2
Rc_AI639197_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx02020 3', mRNA sequence [Rattus norvegicus]	706	904	379	388	2.1
Rc_AI639236_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rz00757 3', mRNA sequence [Rattus norvegicus]	642	653	232	280	-2.5
Rc_AI639313_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx04777 3', mRNA sequence [Rattus norvegicus]	581	667	191	154	3.1
Rc_H31420_at	rc_H31420 EST105436 Rattus norvegicus cDNA, 3' end / clone = RPCAJ34 / clone_end = 3' / gb = H31420 / gi = 976837 / ug = Rn.8443 / len = 312	649	751	1229	1569	-2.0
S54212_at	S54212 ciliary neurotrophic factor receptor alpha component [rats, brain, mRNA, 1332 nt]	302	414	205	209	1.7

Table 11 (cont'd)

Experiment	Description	1-week withdrawal B	1-week withdrawal A	1-week extinction B	1-week extinction A	Fold change
U20283_at	U20283 Rattus norvegicus syntaxin binding protein Munc18-2 mRNA, complete cds / cds= 6,1790 / gb = U20283 / gi = 1022680 / ug = Rn.10121 / len = 2118	206	149	456	442	-2.2
U35774_at	U35774 Rattus norvegicus cytosolic branch chain aminotransferase mRNA, complete cds / cds = 62,1297 / gb = U35774 / gi = 1173633 / ug = Rn.8273 / len = 1370	524	396	245	284	1.7
U36773_at	U36773 RNU36773 Rattus norvegicus glycerol-3-phosphate acyltransferase mRNA, nuclear gene encoding mitochondrial protein, partial cds	134	143	411	549	-2.4
U37101_at	U37101 RRU37101 Rattus rattus granulocyte colony stimulating factor mRNA, complete cds	436	403	59	179	2.1
U50185_g_at	U50185 RNU50185 Rattus norvegicus kidney protein phosphatase 1 myosin binding subunit mRNA, partial cds	345	446	229	197	1.8
U84402_at	U84402 RNU84402 Rattus norvegicus smoothened mRNA, complete cds	537	611	256	219	2.4
U92284_at	U92284 Rattus norvegicus GABA-A receptor epsilon subunit gene, partial cds / cds = 0,1154 / gb = U92284 / gi = 2735328 / ug = Rn.10869 / len = 1600	216	210	78	72	-2.9
X14848cds#12_at	X14848cds#12 MIRNXX Rattus norvegicus mitochondrial genome	461	379	218	221	1.9
X56325mRNA_s_at	X56325mRNA RN2A1GL R.norvegicus 2-alpha-1 globin gene	2029	1486	1069	976	1.7
X58294_at	X58294 R.norvegicus mRNA for carbonic anhydrase II / cds = 8,790 / gb = X58294 / gi = 55837 / ug = Rn.3525 / len = 1459	88	254	387	426	-1.8
X62086mRNA_s_at	X62086mRNA RNCYP3A1 R.norvegicus CYP3A1 gene for cytochrome P450 PCN1	236	254	433	599	-2.1
X69903_at	X69903 R.norvegicus mRNA for interleukin 4 receptor / cds = 9,2411 / gb = X69903 / gi = 56390 / ug = Rn.10471 / len = 2450	417	408	146	118	-3.1
X89968_g_at	X89968 RNSNAPGEN Rattus norvegicus mRNA for alpha-soluble NSF attachment protein	471	555	929	1080	-2.0

[146] Genes that passed the filtering criteria outlined above for differential expression between 1 week extinction and its corresponding control in the medial prefrontal cortex (mPFC). Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 12. mPFC Fold Change 1-Week Withdrawal to Control

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
AB006450_at	AB006450 Rattus norvegicus mRNA for Tim17, complete cds / cds = 4,519 / gb = AB006450 / gi = 2335036 / ug = Rn.2099 / len = 944	167	198	409	326	1.8
AB020504_at	AB020504 Rattus norvegicus mRNA for PMF31, complete cds	181	202	385	361	2.0
AF001898_at	AF001898 Rattus norvegicus aldehyde dehydrogenase (ALDH) mRNA, complete cds / cds = 28,1533 / gb = AF001898 / gi = 2183216 / ug = Rn.6132 / len = 2095	796	993	518	453	-1.8
AF091566_f_at	AF091566 Rattus norvegicus isolate HTF-SP1 olfactory receptor mRNA, partial cds	-151	-48	407	303	1.8
D28111_at	D28111 RATMAOBP2 Rat mRNA for MOBP (myelin-associated oligodendrocytic basic protein), complete cds, clone rOP1	796	860	210	220	3.9
D28560_at	D28560 RATNPHIII Rat mRNA for phosphodiesterase I	420	393	181	272	-1.7
K00512_at	K00512 rat myelin basic protein (mbp) gene mrna / cds = UNKNOWN / gb = K00512 / gi = 205320 / ug = Rn.9672 / len = 1464	3097	3177	696	689	4.5
L13202_f_at	L13202 RATHFH2 Rattus norvegicus HNF-3/fork-head homolog-2 (HFH-2) mRNA, complete cds	84	79	208	198	2.5
L16532_at	L16532 Rattus norvegicus (clone pCNPII) 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPII) mRNA, complete cds / cds = 79,1341 / gb = L16532 / gi = 294526 / ug = Rn.2592 / len = 2301	867	945	244	233	-3.8
L19180_at	L19180 Rat receptor-linked protein tyrosine phosphatase (PTP-P1) mRNA, complete cds / cds = 30,4517 / gb = L19180 / gi = 310201 / ug = Rn.17237 / len = 5396	331	429	46	-13	-1.9
M11794cds#2_f_at	M11794cds#2 RATMT12C Rat metallothionein-2 and metallothionein-1 genes, complete cds	543	480	894	888	1.7
M13100cds#1_g_at	M13100cds#1 RATLIN3A Rat long interspersed repetitive DNA sequence LINE3 (L1Rn)	723	666	1527	1501	2.2
M13100cds#1_at	M13100cds#1 RATLIN3A Rat long interspersed repetitive DNA sequence LINE3 (L1Rn)	1805	1436	2832	2939	1.8
M13100cds#1_g_at	M13100cds#1 RATLIN3A Rat long interspersed repetitive DNA sequence LINE3 (L1Rn)	666	723	1501	1527	2.2
M13100cds#5_s_at	M13100cds#5 RATLIN3A Rat long interspersed repetitive DNA sequence LINE3 (L1Rn)	511	669	1125	1442	2.2
M20721_f_at	M20721 RATPRPA Rat proline-rich protein (PRP-1) mRNA, partial cds	129	100	282	281	2.5
M25888_at	M25888 Rat lipophilin mRNA, 3' end / cds = 0,520 / gb = M25888 / gi = 206223 / ug = Rn.4550 / len = 2585	4308	3199	1042	1483	-3.0

Table 12 (cont'd)

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
M36317_s_at	M36317 RATTRHA Rat thyrotropin-releasing hormone (TRH) precursor mRNA, complete cds	116	132	298	310	2.5
M60322_at	M60322 Rat aldose reductase gene, complete cds / cds = 38,988 / gb = M60322 / gi = 202851 / ug = Rn.2917 / len = 1339	-111	168	562	464	2.6
M80570_at	M80570 Rat dopamine transporter mRNA, complete cds / cds = 62,1921 / gb = M80570 / gi = 310097 / ug = Rn.10093 / len = 3386	491	387	155	80	-2.2
Rc_AI639204_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx03840 3', mRNA sequence [Rattus norvegicus]	309	311	484	606	1.8
Rc_AI639504_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx04791 3', mRNA sequence [Rattus norvegicus]	150	151	297	274	1.9
Rc_AA799448_g_at	rc_AA799448 EST188945 Rattus norvegicus cDNA, 3' end / clone = RHEAB18 / clone_end = 3' / gb = AA799448 / gi = 2862403 / ug = Rn.8296 / len = 615	410	386	197	171	2.2
Rc_AA800604_g_at	rc_AA800604 EST190101 Rattus norvegicus cDNA, 3' end / clone = RLUAB65 / clone_end = 3' / gb = AA800604 / gi = 2863559 / ug = Rn.8590 / len = 579	119	232	396	413	1.9
Rc_AA800693_g_at	rc_AA800693 EST190190 Rattus norvegicus cDNA, 3' end / clone = RLUAK36 / clone_end = 3' / gb = AA800693 / gi = 2863648 / ug = Rn.6620 / len = 533	749	985	553	441	-1.7
Rc_AA818072_s_at	rc_AA818072 UI-R-A0-ag-b-06-0-UI.s2 Rattus norvegicus cDNA, 3' end / clone = UI-R-A0-ag-b-06-0-UI / clone_end = 3' / gb = AA818072 / gi = 2887952 / ug = Rn.11722 / len = 408	440	453	178	228	-2.1
Rc_AA859643_at	rc_AA859643 UI-R-E0-bs-a-08-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-bs-a-08-0-UI / clone_end = 3' / gb = AA859643 / gi = 2949163 / ug = Rn.32 / len = 482	404	520	193	215	-2.2
Rc_AA859922_at	rc_AA859922 UI-R-E0-cg-c-04-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cg-c-04-0-UI / clone_end = 3' / gb = AA859922 / gi = 2949442 / ug = Rn.819 / len = 373	344	413	712	615	1.8
Rc_AA866432_at	rc_AA866432 UI-R-E0-ch-e-06-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-ch-e-06-0-UI / clone_end = 3' / gb = AA866432 / gi = 2961893 / ug = Rn.3106 / len = 484	628	537	302	251	-2.1
Rc_AA875411_s_at	rc_AA875411 UI-R-E0-cs-b-11-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cs-b-11-0-UI / clone_end = 3' / gb = AA875411 / gi = 2980359 / ug = Rn.2911 / len = 423	520	476	191	115	-2.5
Rc_AA875414_at	rc_AA875414 UI-R-E0-cs-d-07-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cs-d-07-0-UI / clone_end = 3' / gb = AA875414 / gi = 2980362 / ug = Rn.2912 / len = 428	218	193	549	634	2.8

Table 12 (cont'd)

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
Rc_AA891940_at	rc_AA891940 EST195743 Rattus norvegicus cDNA, 3' end / clone = RKIAI82 / clone_end = 3' / gb = AA891940 / gi = 3018819 / ug = Rn.3508 / len = 523	427	372	72	143	-2.0
Rc_AA892942_at	rc_AA892942 EST196745 Rattus norvegicus cDNA, 3' end / clone = RKIBA19 / clone_end = 3' / gb = AA892942 / gi = 3019821 / ug = Rn.3611 / len = 511	208	192	85	93	2.3
Rc_AA893593_g_at	rc_AA893593 EST197396 Rattus norvegicus cDNA, 3' end / clone = RPLAC35 / clone_end = 3' / gb = AA893593 / gi = 3020472 / ug = Rn.2272 / len = 443	357	433	59	-12	-2.0
Rc_AA945589_at	rc_AA945589 EST201088 Rattus norvegicus cDNA, 3' end / clone = RLIAP44 / clone_end = 3' / gb = AA945589 / ug = Rn.2151 / len = 569	362	399	860	847	2.2
Rc_AA946313_s_at	rc_AA946313 EST201812 Rattus norvegicus cDNA, 3' end / clone = RLUBD62 / clone_end = 3' / gb = AA946313 / ug = Rn.4295 / len = 505	814	939	445	586	-1.7
Rc_AI070277_s_at	rc_AI070277 UI-R-Y0-ls-h-11-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-Y0-ls-h-11-0-UI / clone_end = 3' / gb = AI070277 / ug = Rn.4550 / len = 355	2415	2557	1169	1337	2.0
Rc_AI072770_s_at	rc_AI072770 UI-R-Y0-md-g-02-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-Y0-md-g-02-0-UI / clone_end = 3' / gb = AI072770 / ug = Rn.4550 / len = 333	1628	1426	327	343	4.6
Rc_H31839_at	rc_H31839 EST106322 Rattus norvegicus cDNA, 3' end / clone = RPCAZ43 / clone_end = 3' / gb = H31839 / gi = 977256 / ug = Rn.14598 / len = 408	292	337	618	681	2.1
U18419_at	U18419 Rattus norvegicus nonmuscle caldesmon mRNA, complete cds / cds = 723,2318 / gb = U18419 / gi = 622966 / ug = Rn.10621 / len = 5541	209	110	372	357	1.8
U31367_at	U31367 Rattus norvegicus myelin protein MVP17 mRNA, complete cds / cds = 75,536 / gb = U31367 / gi = 914967 / ug = Rn.10174 / len = 2268	488	430	192	238	-2.1
U31866_g_at	U31866 Rattus norvegicus Nclone10 mRNA / cds = UNKNOWN / gb = U31866 / gi = 1216376 / ug = Rn.11164 / len = 2657	454	362	111	125	-2.0
U36482_g_at	U36482 Rattus norvegicus endoplasmic reticulum protein ERp29 precursor, mRNA, complete cds / cds = 43,825 / gb = U36482 / gi = 2317799 / ug = Rn.11262 / len = 1115	297	400	126	192	-1.7
U37101_at	U37101 RRU37101 Rattus rattus granulocyte colony stimulating factor mRNA, complete cds	167	85	403	436	2.1
U50185_g_at	U50185 RNU50185 Rattus norvegicus kidney protein phosphatase 1 myosin binding subunit mRNA, partial cds	249	182	446	345	1.8
U89514_at	U89514 Rattus norvegicus calpain large subunit (nCL-4) mRNA, partial cds / cds = 0,2024 / gb = U89514 / gi = 2358261 / ug = Rn.10804 / len = 2195	219	173	484	350	2.0

Table 12 (cont'd)

Experiment	Description	1-week withdrawal control A	1-week withdrawal control B	1-week withdrawal A	1-week withdrawal B	Fold change
X05472cds#1_s_at	X05472cds#1 RNREP24R Rat 2.4 kb repeat DNA right terminal region	1168	971	1824	2461	2.0
X58294_at	X58294 R.norvegicus mRNA for carbonic anhydrase II / cds = 8,790 / gb = X58294 / gi = 55837 / ug = Rn.3525 / len = 1459	626	592	254	88	-2.7
X61295cds_s_at	X61295cds RNL1RTO2B R.norvegicus L1 retroposon, ORF2 mRNA (partial)	1759	2259	3785	4678	2.1
X62086mRNA_s_at	X62086mRNA RNCYP3A1 R.norvegicus CYP3A1 gene for cytochrome P450 PCN1	562	538	254	236	-2.2
X69903_at	X69903 R.norvegicus mRNA for interleukin 4 receptor / cds = 9,2411 / gb = X69903 / gi = 56390 / ug = Rn.10471 / len = 2450	255	162	408	417	1.8
X89968_g_at	X89968 RNSNAPGEN Rattus norvegicus mRNA for alpha-soluble NSF attachment protein	928	1171	555	471	-2.0
Y12502cds_at	Y12502cds RNFXIIIA R.norvegicus mRNA for factor XIIIa	242	225	85	66	3.1
Y13381cds_at	Y13381cds RNAMPH1 Rattus norvegicus mRNA for amphiphysin, amph1	93	73	266	240	3.1

[147] Genes that passed the filtering criteria outlined above for differential expression between 1 week withdrawal and its corresponding control in the medial prefrontal cortex (mPFC). Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 13. VTA 1-Week Extinction to Control

Probe set no.	Description	1-week extinction control A	1-week extinction control B	1-week extinction B	1-week extinction A	Mean control	Mean exp	Ratio	Fold change
AF078779_g_at	AF078779 Rattus norvegicus putative four repeat ion channel mRNA, complete cds	652	476	277	381	652	376.5	0.577454	-1.7
D17614_at	D17614 Rat mRNA for 14-3-3 protein theta-subtype, complete cds / cds = 85,822 / gb = D17614 / gi = 402508 / ug = Rn.2502 / len = 2099	1249	956	407	344	1249	681.5	0.545637	-1.8
rc_AA799299_at	rc_AA799299 EST188796 Rattus norvegicus cDNA, 3' end / clone = RHEAA18 / clone_end = 3' / gb = AA799299 / gi = 2862254 / ug = Rn.8563 / len = 506	70	274	542	430	70	408	5.828571	5.8
rc_AA893191_at	rc_AA893191 EST196994 Rattus norvegicus cDNA, 3' end / clone = RKIBD35 / clone_end = 3' / gb = AA893191 / gi = 3020070 / ug = Rn.3301 / len = 654	55	62	292	313	55	177	3.218182	3.2
rc_AA893327_s_at	rc_AA893327 EST197130 Rattus norvegicus cDNA, 3' end / clone = RKIBF13 / clone_end = 3' / gb = AA893327 / gi = 3020206 / ug = Rn.2732 / len = 452	58	164	354	429	58	259	4.465517	4.5
rc_AA893870_at	rc_AA893870 EST197673 Rattus norvegicus cDNA, 3' end / clone = RPLAM86 / clone_end = 3' / gb = AA893870 / gi = 3020749 / ug = Rn.11229 / len = 417	1935	2636	3948	4037	1935	3292	1.701292	1.7
rc_AA894330_s_at	rc_AA894330 EST198133 Rattus norvegicus cDNA, 3' end / clone = RSPAW76 / clone_end = 3' / gb = AA894330 / gi = 3021209 / ug = Rn.122 / len = 501	657	469	171	236	657	320	0.487062	-2.1
rc_AA944856_at	rc_AA944856 EST200355 Rattus norvegicus cDNA, 3' end / clone = REMAQ02 / clone_end = 3' / gb = AA944856 / gi = 3104772 / ug = Rn.4992 / len = 339	489	381	187	206	489	284	0.580777	-1.7
rc_AI137583_at	rc_AI137583 UI-R-C0-hf-a-03-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C0-hf-a-03-0-UI / clone_end = 3' / gb = AI137583 / ug = Rn.3272 / len = 496	603	482	246	226	603	364	0.603648	-1.7
rc_H31887_at	rc_H31887 EST106421 Rattus norvegicus cDNA, 3' end / clone = RPCBC38 / clone_end = 3' / gb = H31887 / gi = 977304 / ug = Rn.14601 / len = 445	573	816	1374	1058	573	1095	1.910995	1.9
S79214cds_s_at	S79214cds type X collagen alpha 1 chain {NC1 domain} [rats, Genomic, 491 nt]	457	395	136	267	457	265.5	0.580963	-1.7

Table 13 (cont'd)

Probe set no.	Description	1-week extinction control A	1-week extinction control B	1-week extinction B	1-week extinction A	Mean control	Mean exp	Ratio	Fold change
S81924_s_at	S81924 Otx1=homeobox [rats, telencephalon, mRNA Partial, 444 nt]	207	211	-7	27	207	102	0.492754	-2.0
U14398_g_at	U14398 Rattus norvegicus synaptotagmin IV homolog mRNA, complete cds / cds = 267,1544 / gb = U14398 / gi = 550453 / ug = Rn.11072 / len = 2060	518	483	93	171	518	288	0.555985	-1.8
U50842_at	U50842 RNU50842 Rattus norvegicus ubiquitin ligase (Nedd4) protein mRNA, partial cds	415	376	102	177	415	239	0.575904	-1.7
U52663mRNA#3_s_at	U52663mRNA#3 RATPAM27 Rattus norvegicus peptidylglycine alpha-amidating monooxygenase (PAM) gene, exon 26	475	361	181	202	475	271	0.570526	-1.8
X57764_s_at	X57764 Rat mRNA for ET-B endothelin receptor / cds = 203,1528 / gb = X57764 / gi = 56122 / ug = Rn.11412 / len = 1892	519	427	173	228	519	300	0.578035	-1.7
X61106cds_at	X61106cds RNORFEP R.norvegicus ORF for P-glycoprotein (3'-most exon) containing epitope for P-glycoprotein monoclonal antibody, C219	229	207	-257	-304	229	-25	-0.10917	9.2
X96437mRNA_at	X96437mRNA RNPRG1 R.norvegicus PRG1 gene	518	481	99	271	518	290	0.559846	-1.8

[148] Genes that passed the filtering criteria outlined above for differential expression between 1 week extinction and its corresponding control in the ventral tegmental area (VTA). Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 14. VTA 1-Week Extinction to Withdrawal

Probe set no.	Description	1-week with-drawal A	1-week with-drawal B	1-week extinction B	1-week extinction A	Mean control	Mean exp	Ratio	Fold change
AF037072_at	AF037072 Rattus norvegicus carbonic anhydrase III (CA3) mRNA, complete cds / cds = 33,815 / gb = AF037072 / gi = 2708635 / ug = Rn.22519 / len = 1053	770	827	298	360	798.5	329	0.412023	-2.4
D86711_at	D86711 D86711 Rattus norvegicus cDNA / gb = D86711 / gi = 1549215 / ug = Rn.4240 / len = 994	243	252	145	139	247.5	142	0.573737	-1.7
D88034_at	D88034 Rattus norvegicus mRNA for peptidylarginine deiminase type III, complete cds / cds = 42,2036 / gb = D88034 / gi = 1644244 / ug = Rn.10658 / len = 3100	61	61	264	303	61	283.5	4.647541	4.6
E02315cds_f_at	E02315cds DNA encoding calmodulin	2260	2240	799	1028	2250	913.5	0.406	-2.5
L14323_at	L14323 Rattus norvegicus phospholipase C-beta1b mRNA, complete alleles / cds = UNKNOWN / gb = L14323 / gi = 294611 / ug = Rn.9741 / len = 7203	467	367	109	190	417	149.5	0.358513	-2.8
Rc_AI639465_f_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx01612 3', mRNA sequence [Rattus norvegicus]	1172	999	466	341	1085.5	403.5	0.371718	-2.7
Rc_AI639392_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx02714 3', mRNA sequence [Rattus norvegicus]	264	247	84	78	255.5	81	0.317025	-3.2
Rc_AA799410_g_at	rc_AA799410 EST188907 Rattus norvegicus cDNA, 3' end / clone = RHEAA81 / clone_end = 3' / gb = AA799410 / gi = 2862365 / ug = Rn.3326 / len = 612	-118	-60	230	232	-89	231	-2.59551	at least 2 fold
Rc_AA894330_s_at	rc_AA894330 EST198133 Rattus norvegicus cDNA, 3' end / clone = RSPAW76 / clone_end = 3' / gb = AA894330 / gi = 3021209 / ug = Rn.122 / len = 501	628	479	171	236	553.5	203.5	0.36766	-2.7
Rc_AA894345_at	rc_AA894345 EST198148 Rattus norvegicus cDNA, 3' end / clone = RSPAZ21 / clone_end = 3' / gb = AA894345 / gi = 3021224 / ug = Rn.13530 / len = 510	1220	1203	2191	2183	1211.5	2187	1.8052	1.8
Rc_AA899253_at	rc_AA899253 UI-R-E0-cz-g-07-0-Ui.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-E0-cz-g-07-0-Ui / clone_end = 3' / gb = AA899253 / gi = 3034607 / ug = Rn.9560 / len = 410	1463	1238	649	682	1350.5	665.5	0.49278	-2.0

Table 14 (cont'd)

Probe set no.	Description	1-week with-drawal A	1-week with-drawal B	1-week extinction B	1-week extinction A	Mean control	Mean exp	Ratio	Fold change
Rc_AI010083 _at	rc_AI010083 EST204534 Rattus norvegicus cDNA, 3' end / clone = RLUBT52 / clone_end = 3' / gb = AI010083 / ug = Rn.2845 / len = 557	1015	1008	614	528	1011.5	571	0.564508	-1.8
Rc_AI137043 _at	rc_AI137043 UI-R-C2p-oj-c-01-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C2p-oj-c-01-0-UI / clone_end = 3' / gb = AI137043 / ug = Rn.22168 / len = 436	204	226	72	77	215	74.5	0.346512	-2.9
Rc_AI137583 _at	rc_AI137583 UI-R-C0-hf-a-03-0-UI.s1 Rattus norvegicus cDNA, 3' end / clone = UI-R-C0-hf-a-03-0-UI / clone_end = 3' / gb = AI137583 / ug = Rn.3272 / len = 496	613	504	246	226	558.5	236	0.42256	-2.4
Rc_AI237592 _at	rc_AI237592 EST234154 Rattus norvegicus cDNA, 3' end / clone = RPLDB22 / clone_end = 3' / gb = AI237592 / ug = Rn.3747 / len = 592	275	263	111	136	269	123.5	0.459108	-2.2
S69316_s_at	S69316 S69315S2 GRP94/endoplasmin {5' and 3' regions} [rats, KNRK cells, mRNA Partial, 195 nt, segment 2 of 2]	764	625	243	242	694.5	242.5	0.349172	-2.9
AFFX_ratb2/X 14115_at	X14115 Rat DNA for B2 repeat (1-12) from gamma crystallin gene cluster.	212	231	43	56	221.5	49.5	0.223476	-4.5
X55298_at	X55298 Rat ribophorin II mRNA / cds = UNKNOWN / gb = X55298 / gi = 57672 / ug = Rn.6863 / len = 2234	164	189	394	396	176.5	395	2.23796	2.2
X61296cds#2 _f_at	X61296cds#2 RNL1RTO2C R.norvegicus L1 retroposon, ORF2 mRNA (partial)	543	574	63	263	558.5	163	0.291853	-3.4
X96437mRNA _at	X96437mRNA RNPRG1 R.norvegicus PRG1 gene	487	457	99	271	472	185	0.391949	-2.6
Z21935cds_at	Z21935cds RNPROKINA R.norvegicus protein kinase rMNK2	359	332	159	176	345.5	167.5	0.484805	-2.1

[149] Genes that passed the filtering criteria outlined above for differential expression between 1 week withdrawal and 1 week extinction in the ventral tegmental area (VTA). Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 15. VTA 1-Week Withdrawal to Control

Probe set no.	Description	R3KJF020 12264VT	R3KJF020 12263VT	R3KJF020 12261VT	R3KJF020 12262VT	Mean control	Mean exp	Ratio	Fold change
AA799389_g_at	AA799389 EST188886 Rattus norvegicus cDNA, 5' end / clone = RHEAA70 / clone_end = 5' / gb = AA799389 / gi = 2862344 / ug = Rn.3788 / len = 588	398	327	661	694	362.5	677.5	1.868966	1.9
AF015305_at	AF015305 Rattus norvegicus equilbrative nitrobenzylthioinosine-insensitive nucleoside transporter mRNA, complete cds / cds = 157,1527 / gb = AF015305 / gi = 2656138 / ug = Rn.7203 / len = 1678	251	358	590	663	304.5	626.5	2.057471	2.1
AF064868_g_at	AF064868 Rattus norvegicus brain-enriched guanylate kinase-associated protein 1 mRNA, complete cds	-125	-223	465	487	-174	476	-2.73563	at least 2 fold
AF079162_at	AF079162 Rattus norvegicus patched (ptc) mRNA, partial cds	124	102	369	471	113	420	3.716814	3.7
D84667_at	D84667 Rattus norvegicus mRNA for phosphatidy-linositol 4-kinase, complete cds	355	430	260	113	392.5	186.5	0.475159	-2.1
J03179_at	J03179 Rat D-binding protein mRNA, complete cds / cds = 367,1344 / gb = J03179 / gi = 203942 / ug = Rn.11274 / len = 1622	252	261	152	142	256.5	147	0.573099	-1.7
J03886_at	J03886 Rat skeletal muscle myosin light chain kinase, complete cds / cds = 59,1891 / gb = J03886 / gi = 205496 / ug = Rn.9685 / len = 2799	670	891	1565	1194	780.5	1379.5	1.767457	1.8
K00750exon#2-3_at	K00750exon#2-3 RATCYC Rat (Sprague-Dawley) cytochrome c nuclear-encoded mitochondrial gene and flanks	903	713	435	418	808	426.5	0.527847	-1.9
L07925_g_at	L07925 RATGNDSA Rattus rattus guanine nucleotide dissociation stimulator for a ras-related GTPase mRNA, complete cds	224	168	495	413	196	454	2.316327	2.3
M33962_g_at	M33962 Rat protein-tyrosine-phospatase (PTPase) mRNA, complete cds / cds = 119,1417 / gb = M33962 / gi = 206496 / ug = Rn.11317 / len = 4127	201	246	420	391	223.5	405.5	1.814318	1.8
M94918mRNA_f_at	M94918mRNA RATBETGLOX Rat beta-globin gene, exons 1-3	1873	1372	3852	3114	1622.5	3483	2.146687	2.1
rc_AI639204_at	Rat mixed-tissue library Rattus norvegicus cDNA clone rx03840 3', mRNA sequence [Rattus norvegicus]	247	175	453	331	211	392	1.85782	1.9

Table 15 (cont'd)

Probe set no.	Description	R3KJF020 12264VT	R3KJF020 12263VT	R3KJF020 12261VT	R3KJF020 12262VT	Mean control	Mean exp	Ratio	Fold change
rc_AA799571_at	rc_AA799571 EST189068 Rattus norvegicus cDNA, 3' end / clone = RHEAC67 / clone_end = 3' / gb = AA799571 / gi = 2862526 / ug = Rn.3458 / len = 541	510	400	105	253	455	179	0.393407	-2.5
rc_AA892154_g_at	rc_AA892154 EST195957 Rattus norvegicus cDNA, 3' end / clone = RKIAN02 / clone_end = 3' / gb = AA892154 / gi = 3019033 / ug = Rn.3279 / len = 386	224	240	75	54	232	64.5	0.278017	-3.6
rc_AA956149_at	rc_AA956149 UI-R-E1-fg-b-03-0-UI.s2 Rattus norvegicus cDNA, 3' end / clone = UI-R-E1-fg-b-03-0-UI / clone_end = 3' / gb = AA956149 / ug = Rn.8930 / len = 471	239	206	570	752	222.5	661	2.970787	3.0
rc_AI179445_at	rc_AI179445 EST223155 Rattus norvegicus cDNA, 3' end / clone = RSPCH43 / clone_end = 3' / gb = AI179445 / ug = Rn.221 / len = 438	248	230	119	127	239	123	0.514644	-1.9
S61973_at	S61973 NMDA receptor glutamate-binding subunit [rats, mRNA, 1742 nt]	2493	1996	1104	1477	2244.5	1290.5	0.574961	-1.7
S72637_s_at	S72637 tumor-suppressive gene [rats, RSV-trans-formed 3Y1 fibroblast cells, SR-3Y1, mRNA, 1788 nt]	279	202	494	433	240.5	463.5	1.927235	1.9
U21720mRNA_at	U21720mRNA RNU21720 Rattus norvegicus clone C201 intestinal epithelium proliferating cell-associated mRNA sequence	276	369	564	576	322.5	570	1.767442	1.8
U88036_at	U88036 Rattus norvegicus brain digoxin carrier protein mRNA, complete cds / cds = 118,2103 / gb = U88036 / gi = 2501807 / ug = Rn.5641 / len = 3622	443	415	214	278	429	246	0.573427	-1.7
X04070_at	X04070 Rat liver mRNA for gap junction protein / cds = 31,882 / gb = X04070 / gi = 56205 / ug = Rn.10444 / len = 1485	296	279	788	683	287.5	735.5	2.558261	2.6
X60351cds_s_at	X60351cds RRLENSABC R.rattus mRNA for alpha B-crystallin (ocular lens tissue)	295	227	516	417	261	466.5	1.787356	1.8
X61106cds_at	X61106cds RNORFEP R.norvegicus ORF for P-glycoprotein (3'-most exon) containing epitope for P-glycoprotein monoclonal antibody, C219	-1	6	262	277	2.5	269.5	107.8	at least 2 fold
X70667cds_at	X70667cds RRMC3RA R.rattus mRNA for melanocortin-3 receptor	239	295	432	555	267	493.5	1.848315	1.8

Genes that passed the filtering criteria outlined above for differential expression between 1 week withdrawal and its corresponding control in the ventral tegmental area (VTA). Average difference values (from GeneChip version 3.2) are listed for each gene from all groups. Affymetrix probe set numbers are listed along with the common name of the genes, if known.

Table 16: Genes that are differentially regulated in various brain regions in response to extinction and withdrawal

Brain Region

Nac core

Affymetrix Probe Set #	1 week withdrawal to control Gene Name
X56729mRNA_at	calpastatin
	1 week extinction to control
	Gene Name
K02248cds_s_at	somatostatin-14
Z11581_at	kainate receptor subunit (ka2)
	1 week extinction to withdrawal
	Gene Name
M25890_at	melanocortin-3 receptor
M92076_at	somatostatin
X06564_at	metabotropic glutamate receptor 3
AF102855_at	NCAM
	synaptic SAPAP-interacting protein

CeA

	1 week withdrawal to control
	Gene Name
AB016160_g_at	GABAB receptor 1c
D83538_g_at	phosphatidylinositol 4-kinase
	1 week extinction to control
	Gene Name
AB016161cds_i_at	GABAB receptor 1d
AF042830_at	tyrosine kinase receptor Ret (c-ret)
E13644cds_s_at	Neurodap-1
	1 week extinction to withdrawal
	Gene Name
M32754cds_s_at	inhibin alpha-subunit
U14192complete_seq_at	vesicular transport factor

VTA

	1 week withdrawal to control
	Gene Name
D84667_at	phosphatidylinositol 4-kinase
M33962_g_at	protein-tyrosine-phospatase (PTPase)

Table 16 (cont'd)

S61973_at	NMDA receptor glutamate-binding subunit
X70667cds_at	melanocortin-3 receptor
	1 week extinction to control
	Gene Name
U14398_g_at	synaptotagmin IV homolog
	1 week extinction to withdrawal
	Gene Name
E02315cds_f_at	calmodulin
Z21935cds_at	protein kinase rMNK2
L14323_at	phospholipase C-beta1b

Frontal Cortex

	1 week withdrawal to control
	Gene Name
D28560_at	phosphodiesterase I
L19180_at	tyrosine phosphatase (PTP-P1)
M80570_at	dopamine transporter
	1 week extinction to control
	Gene Name
D30040_at	RAC protein kinase alpha
K01701_at	oxytocin/neurophysin
M32061_at	alpha-2B-adrenergic receptor
U56261_s_at	SNAP-25a
	1 week extinction to withdrawal
	Gene Name
D13212_s_at	NMDAR2C
K01701_at	oxytocin/neurophysin
U92284_at	GABA-A receptor epsilon

Nac shell

AJ011318.1	**GABA-B receptor subunit gb2**
X87900.1	Myelin-associated basic protein
L13041.1	Calcitonin receptor
U92535.1	Bos taurus-like neuronal axonal protein
X98051.1	**FRA-2**
AI009098	Similar to human oxygen regulated protein
AI014091	Similar to mouse mrg1 protein
U18772	Pentraxin
U03414	Olfactomedin related protein
U19866.1	Arc – growth factor enriched in dendrites
U28938	Protein tyrosine phosphatase
U67863.1	**Melanocortin 4 receptor**
U69702.1	ALK-7 kinase
U88958.1	Neuritin
X55812.1	**CB1 cannabinoid receptor**

Example 2
Analysis of Western Blots

[150] Figure 7 demonstrates that protein levels of gb2 are increased in the nucleus accumbens shell of the 1 week extinction group compared to control animals. This result supports the microarray results and gives stronger evidence for the role of this protein in drug-seeking. In contrast CB-1 protein levels are increased in the nucleus accumbens of the 1 week withdrawal group compared to controls (Figures 8-10), though the microarray results showed a decrease. Nevertheless, the results suggest an important role for CB-1 in drug-seeking.

[151] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.